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THE UNIVERSITY OF ALBERTA

FIELD AND LABORATORY STUDIES OF DAPHNIA SCHÖDLERI SARS  
FROM BIG ISLAND LAKE, ALBERTA

by



CHI-HSIANG LEI

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES  
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE  
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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Field and Laboratory Studies of Daphnia schødleri from Big Island Lake, Alberta", submitted by Chi-hsiang Lei in partial fulfilment of the requirements for the degree of Master of Science.



## ABSTRACT

The life history of Daphnia schødleri of Big Island Lake, Alberta was investigated by combining field and laboratory studies. Populations of Daphnia schødleri and other zooplankters in Big Island Lake were studied from June 1966 through July 1967. The field study was mainly concerned with seasonal changes of the population density of Daphnia schødleri, and seasonal changes in the species' reproduction.

Extensive winter stagnation in Big Island Lake influenced the seasonal life cycle of Daphnia schødleri. Daphnia schødleri overwintered in the resting egg stage; hatching occurred in late spring and by May parthenogenetic reproduction was taking place. Daphnia schødleri had an annual unimodal curve in respect to population density, with maximum numbers in early summer. By early November, free swimming Daphnia had disappeared from the lake plankton.

Two periods of sexual reproduction occurred in 1966; a major period in June, and a minor period in September. A significant decrease in parthenogenetic egg production (i.e. average brood size) occurred immediately before the onset of the sexual periods, manifesting the state of depression that occurs in Daphnia populations during the transition from parthenogenesis to gamogenesis.

Daphnia used for laboratory studies were reared individually in diluted Banta's manure-soil medium at 22-29.4 C,  $20 \pm 1$  C, and  $5 \pm 1$  C. Daily and sometimes hourly observations were made to study various aspects of Daphnia schødleri's life history, especially growth, longevity, and reproduction.

The number of pre-adult instars of female Daphnia schødleri in culture varied from 4 to 7, with low temperatures increasing the number





of pre-adult instars. At temperatures fluctuating between 22 and 29.4 C, the average longevity was approximately 40.97 days (17.65 instars) for males and 36.42 days (18.21 instars) for females; at  $20 \pm 1$  C, the average longevity for females was 51.93 days (20.78 instars).

Frequency of molting, duration of embryonic development, growth rate, and physiological life span were all influenced by temperature. Low temperatures increased the duration of embryonic development and life span, decreased the frequency of molting, and impeded the growth rate. The production of broods occurred on the average every 2.72 days at  $20 \pm 1$  C and every 18.07 days at  $5 \pm 1$  C. Low temperatures also delayed the onset of sexual maturity of female Daphnia schødleri.

A description is given of the sequential events during embryonic development in vitro, from the 3-hour stage to the 57-hour stage, as observed under microscope.



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## INTRODUCTION

Populations of Daphnia in nature, depending on various environmental conditions, may propagate exclusively by parthenogenesis throughout the year, with all generations of the population consisting only of females; or the population may alternate between parthenogenetic and gamogenetic reproduction during the year, i.e. cyclic reproduction occurs. In the latter case, the populations may overwinter either as adults or as resting eggs enclosed in ephippia. Ephippia can withstand desiccation and freezing, and hence are well suited for carrying the population through periods of unfavourable conditions. The ephippia eventually hatch and give rise to a population consisting of females that reproduce parthenogenetically, the eggs being diploid. Eventually, and depending on factors not yet clearly understood, some of the parthenogenetic eggs develop into males, and then some or all the females reproduce gamogenetically (sexually), producing haploid eggs that require fertilization. These eggs, after fertilization, pass into a modified brood pouch that darkens, eventually becoming black, and then separates from the rest of the carapace to form the ephippium. The eggs, usually two, within the ephippium are sometimes called resting eggs.

Some arctic populations of Daphnia produce ephippia and resting eggs that are apparently not fertilized, since males have not been found in these populations (Edmondson, 1955). Females that produce ephippia may return to parthenogenetic reproduction if conditions improve, or they may produce ephippia again if adverse conditions persist. Besides the mode of reproduction in Daphnia being subject to seasonal changes, the population of Daphnia in lakes, pond or other aquatic habitats, may show none, one, two or more maximum peaks during the course of a year. Thus seasonal life history and seasonal abundance of Daphnia are greatly influenced by the



conditions under which these animals live. In other words, Daphnia is in close harmony with the environmental conditions of a particular habitat.

Many laboratory investigations of Daphnia have been reported: e.g. Banta, Wood, Brown, and Ingle (1939) on the physiology and genetics; Berg (1931, 1934, and 1936), Green (1954, 1956) on reproduction; MacArthur and Baillie (1929), Anderson (1932), Ingle (1933), Anderson, Lumer and Zupancic (1937), Ingle, Wood and Banta (1937), Anderson and Jenkins (1942), LeSuer (1960) concerning laboratory studies of life histories; Coker (1939), Brooks (1946), Hazelwood (1962, 1966) on cyclomorphosis; Pratt (1943), Slobodkin (1954), Frank (1960) concerning laboratory studies on population development; Obreshkove and Fraser (1940) on the embryonic development of parthenogenetic eggs; Fox (1948), Fox, Gilchrist and Phear (1951), Chandler (1954), Green (1955) on haemoglobin of Daphnia.

Numerous studies of seasonal life history and succession of various species of Daphnia also have been carried out in various regions of the world: e.g. Birge (1898), Brooks (1946), Edmondson (1955), Hall (1964) in America; Green (1955), Elbourn (1966) in Great Britain; Wesenberg-Lund (1904), Berg (1931) in Denmark; Wiktor (1961) in Poland; Wagler (1912) in Germany.

Daphnia schødleri Sars is predominantly an inhabitant of ponds and small lakes in North America. The species was first described by G. O. Sars in 1862, but subsequently was called a variety or subspecies of Daphnia pulex by different workers. Woltereck (1932) described it as Daphnia pulex pulicoides. Brooks (1957), in his revision of the genus Daphnia, recognized it as a distinct species, D. schødleri.

The present study was undertaken to investigate the seasonal events of D. schødleri in Big Island Lake, Alberta. This lake is located in a region of long, cold winters and cool, short summers. The lake exhibits long periods of ice cover, and winter stagnation occurs before the





break-up. Seasonal life history, seasonal variation in egg production, and cyclic reproduction were investigated. Laboratory studies on various aspects of D. schødleri's life history were also made. These studies included growth, mortality, and reproduction.



## DESCRIPTION OF STUDY AREA

Big Island Lake is a shallow, unstratified eutrophic lake (Fig. 1). It is located approximately 17 miles southeast of Edmonton, Alberta. The surface area is about 300 acres (121.4 hectares), with a maximum depth of about 2.5 meters. Most of the lake is less than 2 meters in depth. An island, located in the middle of the lake, covers an area of nearly 12 acres (4.8 hectares). The entire shoreline is surrounded by emergent vegetation, chiefly cattail, Typha latifolia L.; reed grass, Phragmites communis Trin.; brome grass, Bromus erectus Huds.; and bur-reed, Sparganium sp. Extending lakeward from the cattail region are extensive growths of Potamogeton Richardsonii (Benn.) and Potamogeton pectinatus L.. Most of the lake has a mud bottom, composed chiefly of autochthonous organic detritus.

During the winter months, Big Island Lake is completely covered by ice. In 1966-1967, ice started forming during the first week of November and remained on the lake until the second week of May. The ice during late winter was about 2 feet thick and covered by one foot of snow. Intense winter stagnation occurred from January through April. In these months, there was no detectable dissolved oxygen in the lake, and  $H_2S$  was present.

Figure 2a shows water temperatures for the 14 month period, June 1966 through July 1967. Water temperatures rose rapidly in the spring after the ice cover had melted, reaching a peak in early July 1966, and then declined gradually until the end of August. Temperatures dropped rapidly beginning in late August, and by 16 October the temperature was 4 C. From November through April, the lake was completely ice-covered, and the average water temperature was 1.5 C. In early May 1967, the ice started breaking up, and water temperature gradually rose.



Figure 1. Outline map of Big Island Lake in July 1965, showing areas covered by open lake, cattail, Potamogeton and sandy shore. (After Menon, 1966.)



Big Island Lake.

Scale : 1 cm. = 0.157 km.

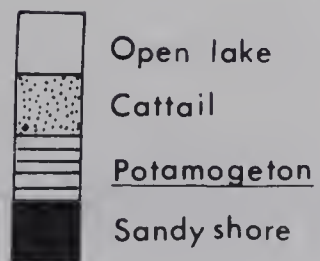
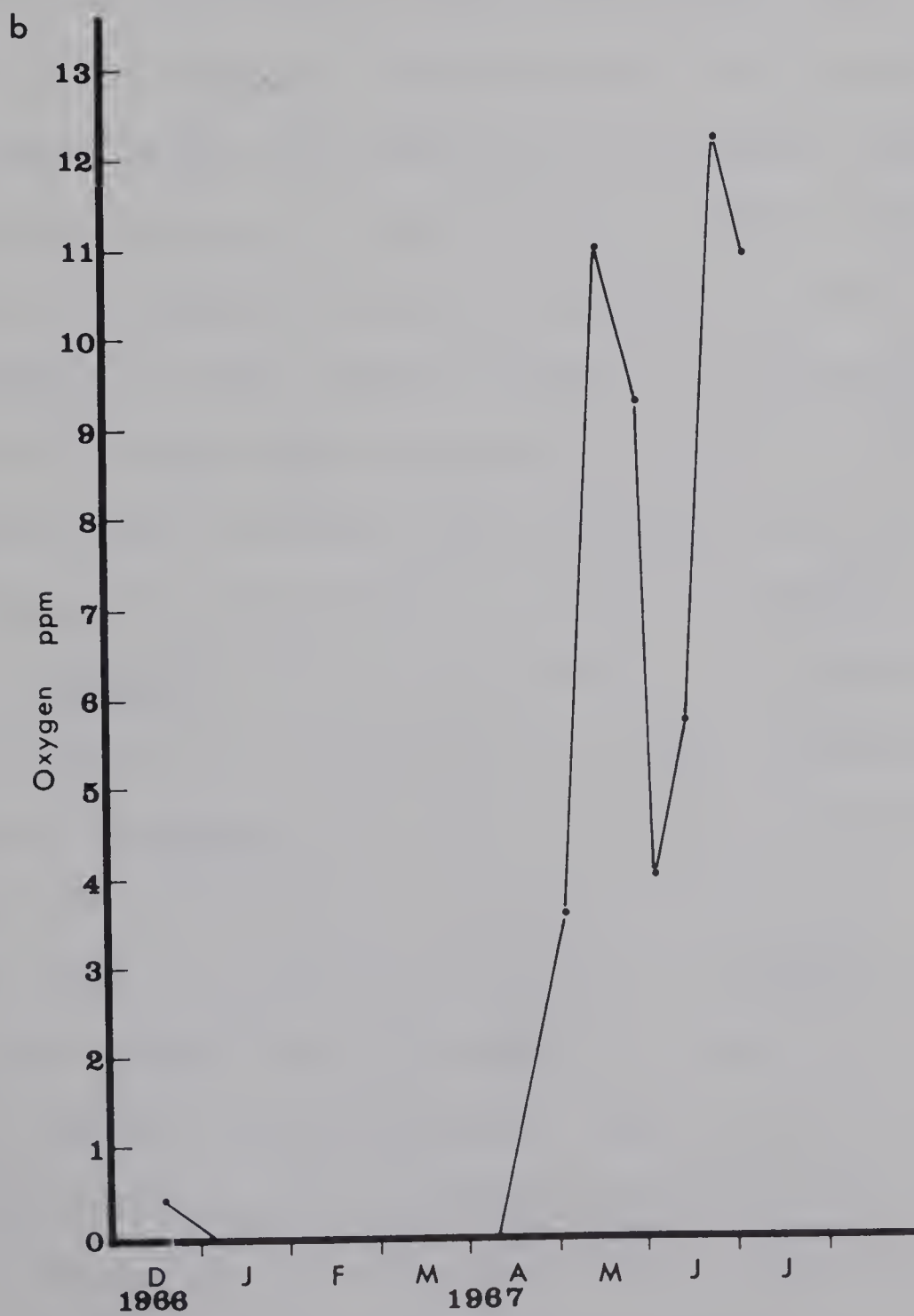
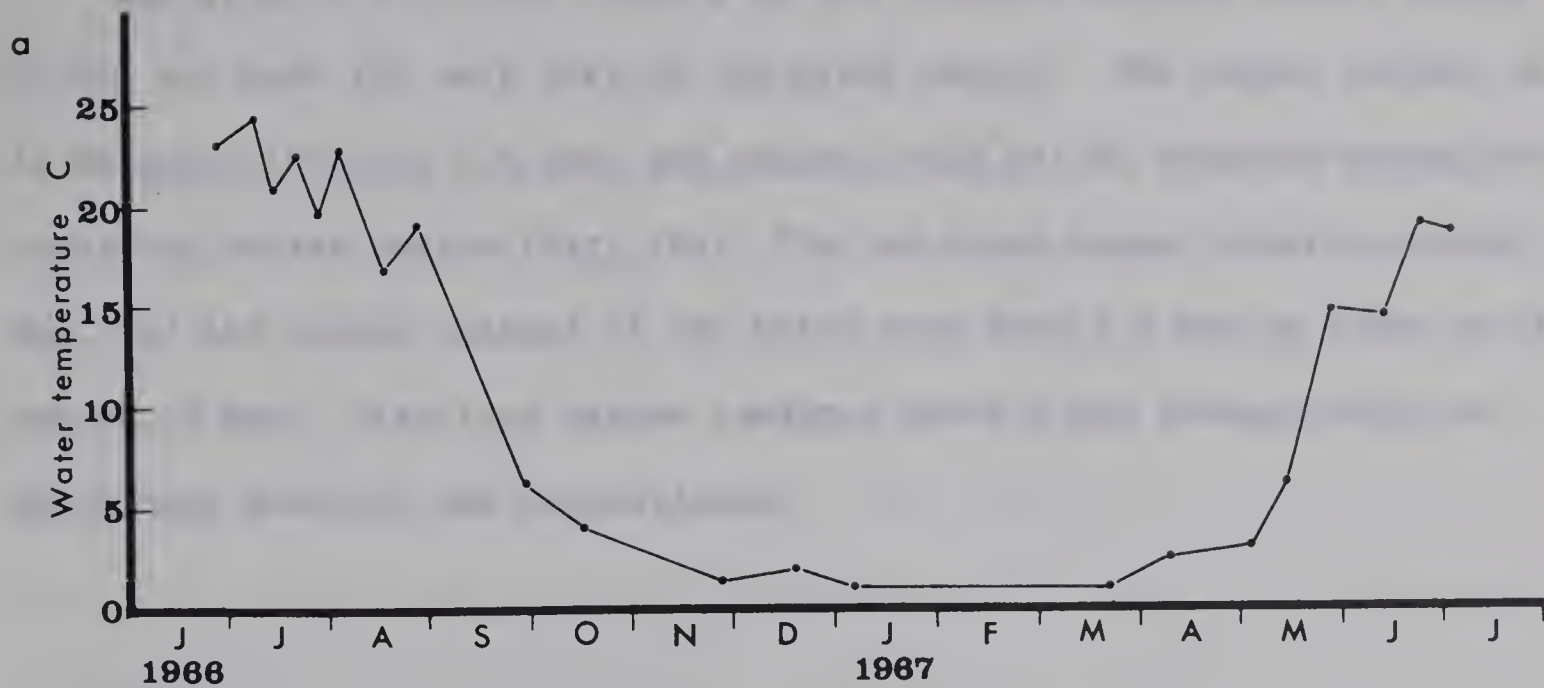


Figure 2a. Water temperatures of Big Island Lake, 29 June 1966  
to 3 July 1967.

Figure 2b. Dissolved oxygen in Big Island Lake, 18 December 1966  
to 3 July 1967.





Analysis of dissolved oxygen, by the standard Winkler method (Welch, 1948), was made for only part of the study period. The oxygen content on 18 December 1966 was 0.4 ppm, and oxygen could not be detected during the remaining winter months (Fig. 2b). The ice cover began to melt in early May, and the oxygen content of the water rose from 3.6 ppm on 3 May to 11 ppm on 13 May. Dissolved oxygen remained above 4 ppm through July, at which time analysis was discontinued.





## METHODS

### Field Studies

Daphnia schødleri was studied in Big Island Lake from 13 June 1966 to 3 July 1967 (Table 1). During the ice-free months, plankton samples were taken from the limnetic region of the lake by hauling a plankton net, having a number 20 mesh size and a 12.5 cm diameter opening, obliquely from near the bottom of the lake to the surface. The plankton net was operated by hand from a small boat, and for each sampling date four hauls were made at randomly chosen sites. During the winter months, plankton samples were collected by dropping the net vertically through a hole in the ice; the net remained on the bottom for 2 minutes, and it then was pulled to the surface.

Plankton samples were preserved in five percent formalin. In the laboratory each sample was then diluted to a known volume (usually 100 to 200 ml), depending upon the number of plankters present in the sample. To count Daphnia and other plankton, several 1 ml subsamples were taken using a wide-mouth pipette. Each subsample was placed in a Sedgwick-Rafter cell and examined under low-power (40x) of a microscope, which was equipped with a mechanical stage and an ocular (or eyepiece) micrometer. In this way, at least 100 Daphnia were counted and measured. For samples containing less than 100 Daphnia, all individuals were counted and measured. Length measurements of Daphnia were made from the top of the head to the base of the spine (Fig. 3); this was designated as body length.

To analyze the population composition of Daphnia, individuals in each sample were grouped into five categories as suggested by Green (1955):

- 1) Females with parthenogenetic eggs or embryos in the brood pouch, or females with large ovaries indicating that eggs were about to be laid.
- 2) Females of mature size (over 54.7 micrometer units = 1.78 mm) but without eggs or large ovaries, and possessing the long abdominal process



Table 1. Sampling program for a study period, June 1966 through July 1967.

Numbers in the parentheses are the times at which samples were taken.

1966										1967		
<u>June</u>	<u>July</u>	<u>Aug.</u>	<u>Sept.</u>	<u>Oct.</u>	<u>Nov.</u>	<u>Dec.</u>	<u>Jan.</u>	<u>Mar.</u>	<u>Apr.</u>	<u>May</u>	<u>June</u>	<u>July</u>
13 (0930)	8 (0945)	3 (1215)	28 (0930)	16 (1000)	27* (1025)	18* (1000)	5* (0950)	19* (1030)	9* (1030)	3 (1500)	3 (1030)	3 (1033)
22 (0930)	13 (0950)	17 (1045)						13 (1055)		13 (1055)	13 (0935)	
29 (1015)	19 (1030)	26 (0946)								27 (1000)	24 (1030)	
	27 (1028)											

\* denotes samples taken through holes in the ice.





with which eggs are retained in the brood pouch. These are females that have become temporarily or permanently sterile.

3) Females with ephippia, or females with the carapace showing signs of ephippial formation and the corresponding appearance of ephippial eggs in the ovary; or with ephippial eggs in the ovary but without showing any signs of ephippial formation. These ovaries are composed of a compact dark mass, representing the developing resting eggs.

4) Immature females. These are smaller than mature females (i.e. smaller than 1.78 mm) and lack the long abdominal process that retains eggs in the brood pouch.

5) Males, either immature or mature specimens.

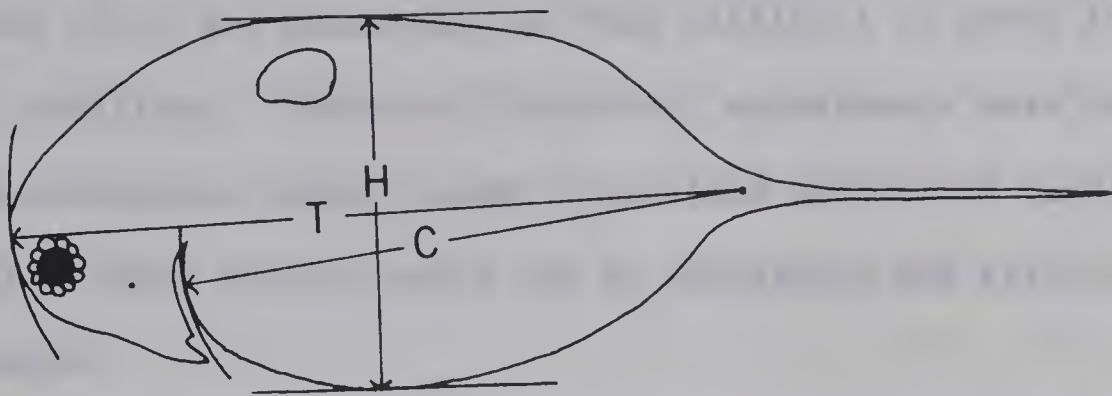
The carapaces of category 1 animals were opened with fine needles, and the number of eggs or embryos in the brood pouches were counted under low-power of a dissecting microscope. Unless otherwise stated, the term "egg" refers to parthenogenetic eggs. The term "egg number" is used irrespectively of whether eggs or embryos were counted. The mean egg number was calculated from a sample of at least 25 females having eggs in their brood pouches.

To estimate the volume of parthenogenetic eggs, samples of at least 50 eggs were dissected out of the brood pouches of females of various sizes, and the measurements were made with the eggs covered in a film of water on a slide. Two diameters were measured, the largest and the smallest. The volume of each egg was then calculated using the formula:

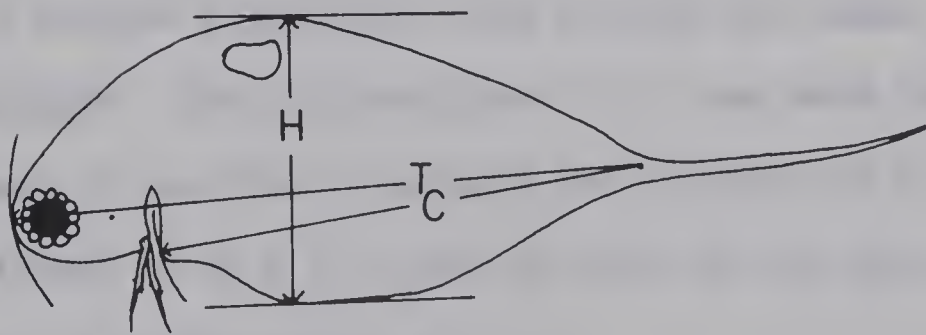
$$V = 1/6 \cdot \pi g l^2$$

where  $g$  is the largest diameter and  $l$  is the smallest. Volumes were determined only for eggs at late stage 1 or early stage 2 of embryonic development (cf. section on laboratory studies for explanation of stages), developing embryos beyond early stage 2 being rejected. Since newly deposited eggs tend to swell when first laid into the brood pouch, these also were rejected.

Figure 3. Daphnia schødleri showing various measurements of males and females. T, total length, which is the longest dimension of animal exclusive of spine; C, carapace length, which is the longest dimension of the carapace exclusive of spine; H, height, which is the shortest distance between two lines tangential to the carapace.



Female (x42)



Male (x38)



The same methods were used to measure the length of ehippial females and to calculate the volumes of resting eggs carried by the females.

### Laboratory Experiments

Many biological aspects of Daphnia, such as longevity, duration of instars, number of pre-adult instars, growth, reproduction, and development of embryos, etc., are impossible or very difficult to study in detail under natural conditions. Therefore laboratory experiments were conducted to study these biological aspects under controlled laboratory conditions. It was hoped that these results would aid in explaining the results obtained from field studies.

Young daphnids were isolated a few hours after being released from the brood pouch. They were measured and were individually placed in a small beaker of 50 ml capacity containing 20-25 ml of culture medium. The culture medium used in this experiment was the diluted medium of Banta's manure-soil stock medium (Banta, 1921). It was prepared by adding one part of stock medium to four parts of filtered aquarium water. On alternate days, the total volume of the medium was replaced with fresh medium.

Four sets of culture experiments were carried out under different temperature conditions. Two sets were kept in a room with temperatures fluctuating between 22 and 29.4 C; another set was kept in a water bath with temperature maintained at  $20 \pm 1$  C; and the last set was kept in a refrigerator with temperature maintained at  $5 \pm 1$  C.

At the time of isolation, and daily thereafter, each individual was placed in a shallow depression slide with one drop of culture medium. One drop of saturated chloretone solution was added to the medium of animals kept at room temperatures (22-29.4 C); this facilitated accurate measurements (by impeding activity). Chloretone was not needed for animals at other temperatures. Measurements as shown in Figure 3 were made with a





calibrated ocular micrometer. Animals kept at room temperatures fluctuating between 22 and 29.4 C were examined at least once a day. Immediately before daily measurements were taken, the presence of cast carapaces, the presence of eggs in the brood pouch, and the number of young released in each brood were noted.

The animals kept at  $20 \pm 1$  C were observed hourly from 0800 to midnight each day, from the time the animals passed as eggs into their mother's brood pouch to the end of their adult life. Records were kept of the time each individual passed as an egg into the brood pouch of the mother, the time of release from the brood pouch as a free living young, the time of each molt, the time of release of her own young, and the number of young produced in each brood.

The daphnids used in the two sets of culture experiments kept at temperatures fluctuating between 22 and 29.4 C were started from a non-ephippial female that was taken from Big Island Lake on 22 June 1966. This female, which contained eggs in the brood pouch when collected, was cultured in finger bowl with the water taken from the lake. She shortly released five young females. Thrity one eggs were then deposited into the brood pouch again. These eggs were dissected out from the brood pouch and put into the depression slide with a few drops of filtered aquarium water. Two days later, 24 active, young males developed from these eggs. Each of the young males was placed into a small beaker of 50 ml capacity with 20 ml of diluted Banta's manure-soil medium and reared individually. The females used in the various experiments were the second generations of the original female collected from Big Island Lake; hence all females and males used in the culture experiments maintained at room temperature fluctuating between 22 and 29.4 C were of the same clone.



For experiments at  $20 \pm 1$  C and  $5 \pm 1$  C, daphnids were started from a non-ephippial female taken from Big Island Lake on 3 July 1967, when the population of Daphnia in the lake was in active gamogenesis. The animals used for culture experiments were the third generation of this non-ephippial female.





## FIELD STUDIES

## Description of the Zooplankton

## of Big Island Lake

Five species of Copepoda were found in the limnetic region of Big Island Lake, with Cyclops varicans rubellus, Cyclops bicuspidatus thomasi, and Diaptomus siciloides being the most abundant species (Table 2).

Cyclopoids were found throughout the year, even in the winter during the period of stagnation and oxygen depletion.

Daphnia schødleri, Diaphanosoma leuchtenbergianum and Bosmina coregoni were the commonest Cladocera in the lake, Chydorus sp. and Ceriodaphnia sp. seldom being encountered in the samples. Specimens of B. coregoni with parthenogenetic eggs were first encountered on 29 June 1966 and 13 June 1967. During October and November 1966, both females with resting eggs and males were found. Diaphanosoma leuchtenbergisnum was first encountered in the 1966 samples on 22 June, and females with parthenogenetic eggs in brood pouches were first collected on 8 July 1966. In 1966, females with resting eggs, were collected in August; males were also collected at this time. In October, females with resting eggs were also encountered, but no males were found. At this time unattached resting eggs of this species were also collected. By 27 November 1966, free swimming Diaphanosoma had completely disappeared from the lake, and they were not encountered again until May 1967.



Table 2. Limnetic zooplankton found in Big Island Lake. x indicates present in sample.

	1966							
	June 13	June 22	June 29	July 8	July 13	July 27	Aug. 3	Aug. 17
<b>Cladocera</b>								
<u>Daphnia schødleri</u> Sars	x	x	x	x	x	x	x	x
<u>Diaphanosoma leuchtenbergianum</u> Fischer	-	-	x	x	x	x	x	x
<u>Bosmina coregoni</u> Baird	x	-	x	x	-	x	x	x
<u>Chydorus</u> sp.	-	-	-	-	-	-	-	-
<u>Ceriodaphnia</u> sp.	x	-	-	-	x	-	-	-
<b>Copepoda</b>								
<u>Cyclops varicans rubellus</u> Lilljeborg	x	x	x	x	x	x	x	x
<u>Cyclops bicuspidatus thomasi</u> Forbes	x	x	x	x	x	x	x	x
<u>Macrocyclus albidus</u> (Jurine)	x	x	x	x	x	x	x	x
<u>Eucyclops agilis</u> (Koch)	x	x	-	x	x	x	-	-
<u>Diaptomus siciloides</u> Lilljeborg	x	x	x	x	x	x	x	x
Nauplii	x	x	x	x	x	x	x	x
<b>Rotifera</b>								
<u>Keratella cochlearis</u> (Gosse)	x	x	x	x	x	x	x	x
<u>Keratella quadrata</u> (O.F.M.)	x	x	x	x	-	x	x	x
<u>Brachionus</u> sp.	x	-	x	x	-	x	x	x
<u>Rotaria neptunia</u> (Ehrenberg)	x	-	x	x	x	x	x	x
<u>Filinia longiseta</u> (Ehrenberg)	x	x	x	x	x	x	x	x
<u>Asplanchna</u> sp.	x	x	-	-	-	-	-	-
<u>Polyarthra</u> sp.	x	-	x	-	-	-	-	-
<u>Trichocerca</u> sp.	-	-	x	x	x	x	-	-
<u>Dipleuchlanis</u> sp.	x	-	-	-	-	-	-	-
<u>Lepadella</u> sp.	-	-	-	-	-	-	-	-
<b>Protozoa</b>								
<u>Vorticella</u> sp.	x	x	x	x	x	x	x	x
<u>Epistylis</u> sp.	-	-	-	-	-	-	x	x
<u>Zoothamnium</u> sp.	-	-	x	-	-	-	-	-
<u>Carchesium</u> sp.	-	-	-	-	-	-	-	-
<u>Diffugia</u> sp.	x	x	-	-	-	x	-	x
<u>Arcella</u> sp.	-	-	-	-	x	-	x	-
Holotricha	x	x	x	x	x	x	x	x





Table 2. (Continued)

1967								
	Aug. 26	Oct. 16	Nov. 27	Dec. 18	Jan. 5	Mar. 19	Apr. 9	May 13
<b>Cladocera</b>								
<u>Daphnia schødleri</u> Sars	x	x	-	-	-	-	-	-
<u>Diaphanosoma leuchtenbergianum</u> Fischer	x	x	-	-	-	-	-	-
<u>Bosmina coregoni</u> Baird	-	x	x	x	-	-	-	-
<u>Chydorus</u> sp.	-	x	x	-	-	-	-	-
<u>Ceriodaphnia</u> sp.	-	-	-	-	-	-	-	-
<b>Copepoda</b>								
<u>Cyclops varicans rubellus</u> Lilljeborg	x	x	x	x	x	x	x	x
<u>Cyclops bicuspidatus thomasi</u> Forbes	x	x	x	x	x	x	x	x
<u>Macrocylops albidus</u> (Jurine)	x	x	-	-	x	x	-	x
<u>Eucyclops agilis</u> (Koch)	x	x	x	x	-	x	-	x
<u>Diaptomus siciloides</u> Lilljeborg	x	x	x	-	-	-	-	-
Nauplii	x	x	x	-	-	-	-	x
<b>Rotifera</b>								
<u>Keratella cochlearis</u> (Gosse)	x	x	x	-	-	-	-	x
<u>Keratella quadrata</u> (O.F.M.)	x	x	x	-	-	-	-	x
<u>Brachionus</u> sp.	x	-	-	-	-	-	-	-
<u>Rotaria neptunia</u> (Ehrenberg)	x	-	x	x	x	x	x	x
<u>Filinia longiseta</u> (Ehrenberg)	x	x	x	-	-	-	-	-
<u>Asplanchna</u> sp.	-	-	-	-	-	-	-	x
<u>Polyarthra</u> sp.	-	-	-	-	-	-	-	-
<u>Trichocerca</u> sp.	-	-	-	-	-	-	-	-
<u>Dipleuchlanis</u> sp.	-	-	-	-	-	-	-	x
<u>Lepadella</u> sp.	-	-	-	-	-	-	-	x
<b>Protozoa</b>								
<u>Vorticella</u> sp.	x	x	x	-	x	x	x	x
<u>Epistylis</u> sp.	-	-	x	-	-	x	x	-
<u>Zoothamnium</u> sp.	-	x	-	-	-	x	x	x
<u>Carchesium</u> sp.	-	x	-	-	-	-	x	-
<u>Diffflugia</u> sp.	x	x	-	x	x	x	x	x
<u>Arcella</u> sp.	-	-	-	-	-	-	-	x
Holotricha	x	x	x	x	x	x	x	x





Table 2. (Continued)

1967					
	May 27	June 3	June 13	June 24	July 3
Cladocera					
<u>Daphnia schødleri</u> Sars	x	x	x	x	x
<u>Diaphanosoma leuchtenbergianum</u> Fischer	x	x	x	x	x
<u>Bosmina coregoni</u> Baird	x	-	x	-	x
<u>Chydorus</u> sp.	x	-	-	-	-
<u>Ceriodaphnia</u> sp.	-	-	-	-	-
Copepoda					
<u>Cyclops varicans rubellus</u> Lilljeborg	x	x	x	x	x
<u>Cyclops bicuspidatus thomasi</u> Forbes	x	x	x	x	x
<u>Macrocyclus albidus</u> (Jurine)	x	x	x	x	x
<u>Eucyclops agilis</u> (Koch)	x	x	x	x	x
<u>Diaptomus siciloides</u> Lilljeborg	-	x	x	x	x
Nauplii	x	x	x	x	x
Rotifera					
<u>Keratella cochlearis</u> (Gosse)	x	x	x	x	x
<u>Keratella quadrata</u> (O.F.M.)	x	x	x	x	x
<u>Brachionus</u> sp.	x	x	x	x	x
<u>Rotaria neptunia</u> (Ehrenberg)	x	x	x	x	x
<u>Filinia longiseta</u> (Ehrenberg)	x	x	x	x	x
<u>Asplanchna</u> sp.	x	-	-	x	-
<u>Polyarthra</u> sp.	-	x	-	x	x
<u>Trichocerca</u> sp.	-	-	-	-	x
<u>Dipleuchlanis</u> sp.	-	-	-	-	-
<u>Lepadella</u> sp.	-	-	-	-	-
Protozoa					
<u>Vorticella</u> sp.	x	x	x	x	x
<u>Epistylis</u> sp.	-	-	x	x	x
<u>Zoothamnium</u> sp.	x	-	-	-	-
<u>Carchesium</u> sp.	x	-	-	-	-
<u>Diffugia</u> sp.	-	-	x	x	x
<u>Arcella</u> sp.	-	-	-	-	-
Holotricha	x	x	x	x	x



## Seasonal Changes in Major Zooplankters

other than D. schodleri

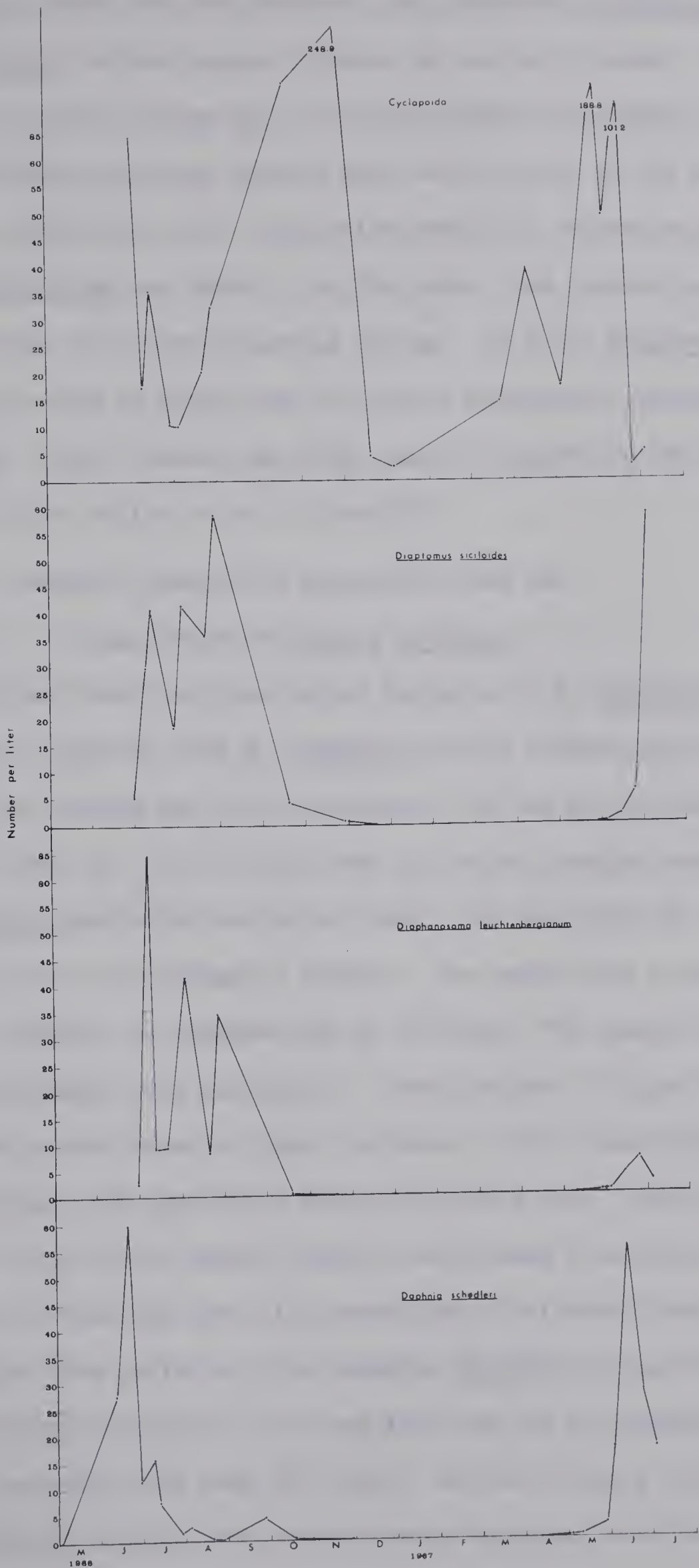
Seasonal changes in the population densities of Diaphanosoma, Diaptomus and Cyclopoida are illustrated in Figure 4. The population densities of Diaptomus and Cyclopoida include both adults and copepodites. The seasonal curve for Cyclopoida represents all species of this order found in the lake, C. varicans rubellus and C. bicuspidatus thomasi being the most abundant species.

Cyclopoids were found throughout the year. The 1966 population reached its peak in November, but during early winter cyclopoids numbers declined rapidly to a minimum during January 1967. From December 1966 through 9 April 1967, all cyclopoids consisted of late copepodites and a few adult females. Apparently reproduction does not take place during the winter months. In 1967 females carrying egg sacs were first collected on 13 May. Males were also collected at this time, and a few nauplii were also present.

Diaptomus siciloides, a calanoid copepod, reproduced almost continuously during the summer months of 1966. Adult females carrying egg sacs or spermatophores were collected from June through mid-October 1966. The population reached its maximum density in late August. Population numbers then fell rapidly and continuously, and by 27 November few D. siciloides were found in the lake. The few remaining specimens were adult males and females, but neither females carrying egg sacs nor females with attached spermatophores were encountered. Nauplii were also absent. By 18 December, Diaptomus (both young and adults) had completely disappeared from the lake and were not found again until the following spring, nauplii first being encountered on 13 May 1967. In 1967, males and adult females carrying egg sacs were first encountered on 13 June. The sex ratio of males and females at this time was 0.5, with 6.7 percent of the females carrying egg sacs.

Figure 4. Seasonal changes in population size (numbers/liter) of Cyclopoida, Diaptomus siciloides, Diaphanosoma leuchtenbergianum, and Daphnia schødléri in Big Island Lake, June 1966 to July 1967.









Unlike the above two zooplankters, the cladoceran Diaphanosoma leuchtenbergianum reached maximum numbers in the early summer. Males, and females carrying resting eggs, were collected during August 1966. On 16 October, females carrying resting eggs were present in the sample. Following the early July peak, population densities decreased, and by 27 November Diaphanosoma was absent from the lake. The species was not encountered again until the following spring. In 1967, Diaphanosoma first appeared in late May at which time the entire population consisted of young females. Adult females carrying eggs and embryos in the brood pouches were first collected on 13 June 1967.

#### Seasonal Changes in Population Size and

#### Composition of Daphnia schødleri

In early May 1966, only unattached ephippia of D. schødleri were found in the samples, implying that D. schødleri in Big Island Lake had overwintered in the resting egg stage (ephippia) and had not yet hatched from the ephippia (Fig. 4). By 13 June, when the second samples were collected, the D. schødleri population was quite large. At this time the entire population consisted of non-ephippial females. The population continued to increase and reached its maximum peak on 22 June. The number of D. schødleri then declined through July and August. From the end of August to 28 September, the population again showed a slight increase. But it soon decreased again and by 16 October, the population density was very low. From 27 November 1966 through 9 April 1967 monthly samples were taken from holes in the ice, and samples of bottom mud were also occasionally collected using a Ekman dredge. During this period no free swimming Daphnia were encountered, except for unattached ephippia. On 3 May 1967 the ice was breaking up and sampling was possible only near the shore. At this time, a few young Daphnia, obviously hatched from overwintering ephippia, were found. Their



size varied from 0.49 to 0.65 mm. By the middle of May, the population was still small, but it increased rapidly and reached a peak on 13 June 1967. The number of D. schødleri then decreased and was continuing to decrease when the study was terminated.

To determine the period of sexual reproduction for D. schødleri, the population, for each sampling date, was separated into five reproductive categories (Table 3). There were two periods of sexual reproduction in 1966. The first started during the last half of June and continued through July, lasting about 5 or 6 weeks. The second period occurred during September. Unfortunately, only one sample was taken between 26 August and 16 October; hence the length of this second period of sexual reproduction is not known. During the first period, the highest percentage of ehippial females was found on 22 June, when the population density was at its peak. During subsequent sampling dates, the percentage of ehippial females declined continuously, and by 3 August, the ehippial females had completely disappeared from the population. Ehippial females occurred only in small number on 28 September, during the second sexual reproduction period.

The percentage of males found during both periods of sexual reproduction was quite low, except on 16 October when males comprised 16.7 percent of the total sample. But in percentage composition analyses, the percentage of one group may increase either because of an absolute increase in that group or because of a decrease in another group. On 16 October both ehippial females and parthenogenetic egg-bearing females had disappeared from the population, and the number of young individuals had also decreased. This would influence the percentage composition of males on this date.







Table 3. Seasonal changes in percentage composition of life cycle stages of Daphnia schøddleri in Big Island Lake, June 1966 to July 1967.

Date	Females with parthenogenetic eggs	Females of mature size without eggs	Immature females	Ephippial females	Males	Number of individuals counted
13 June (1966)	6.3	2.6	91.1	0	0	304
22 June	13.2	14.1	29.2	43.9	0.6	319
29 June	9.3	21.8	28.0	40.9	0	193
8 July	7.4	0.7	87.4	4.1	0.4	269
13 July	11.4	2.2	85.9	0.5	0	185
19 July	44.2	10.9	41.0	3.9	0	129
27 July	17.5	2.9	79.0	0.6	0	68
3 Aug.	1.9	0	98.1	0	0	103
17 Aug.	19.4	9.7	70.9	0	0	31
26 Aug.	20.0	20.0	60.0	0	0	5
28 Sept.	15.0	18.0	63.0	3.7	0.3	85
16 Oct.	0	33.3	50.0	0	16.7	6
27 Nov.	0	0	0	0	0	0
18 Dec.	0	0	0	0	0	0
5 Jan. (1967)	0	0	0	0	0	0
19 Mar.	0	0	0	0	0	0
9 Apr.	0	0	0	0	0	0
3 May	0	0	100.0	0	0	5
13 May	0	0	100.0	0	0	8
27 May	16.2	0.8	83.0	0	0	130
3 June	20.4	5.6	73.6	0	0.4	284
13 June	5.9	4.0	71.6	18.4	0.1	1287
24 June	13.5	11.0	56.3	18.9	0.3	318
3 July	7.6	5.5	75.9	7.2	3.8	237



In brief, the population of D. schødleri in Big Island Lake overwintered as resting eggs (ephippia), and hatched from resting eggs in the early spring (May) of the following year. Soon after hatching, the population rapidly increased in numbers via a series of parthenogenetic generations. The population reached its peak density in June. Following this increase, the reproductive rate (egg production) of female D. schødleri began to decrease, females began to change from parthenogenetic reproduction to bisexual reproduction, and males and ephippial females appearing in the population. With the intervention of sexual reproduction, the population of D. schødleri decreased again, and it reached minimum numbers at the end of August. Following the decrease in population density, D. schødleri reverted to parthenogenetic reproduction and showed a slight increase in population numbers during early September. After this slight increase, a second period of sexual reproduction took place. D. schødleri began to decrease in number again, and eventually completely disappeared from the lake plankton in the early November. Thus D. schødleri overwintered as resting eggs, which were produced during two periods of sexual reproduction.

#### Seasonal Changes in Length-frequency

#### Distributions of D. schødleri

Knowledge of the age and size structure of a metazoan population is necessary to analyze properly its population growth (Slobodkin, 1954). Planktonic crustaceans including Daphnia exhibit no age-specific characters, making it difficult to describe the age structure of the population. Since the size of a Daphnia of a given age depends, at least in part, on its nutritional history, the age of an individual does not predict its size (or vice versa), unless its nutritional history is also known. However, some understanding of population growth can be obtained by separating the





Daphnia population into arbitrarily selected size classes, and following these size classes throughout the year.

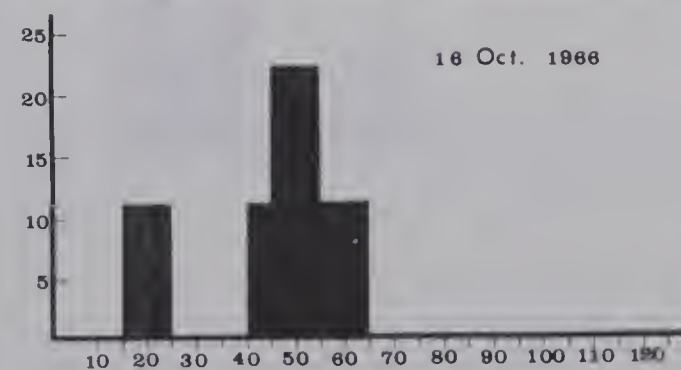
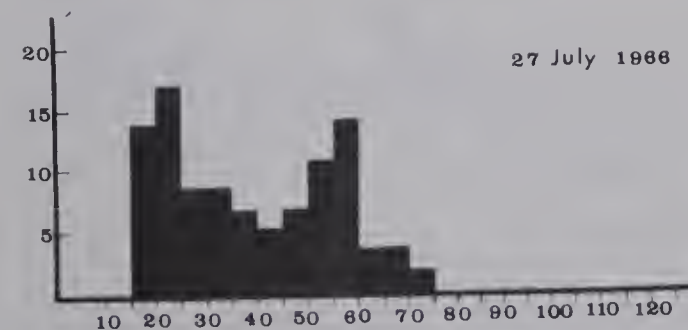
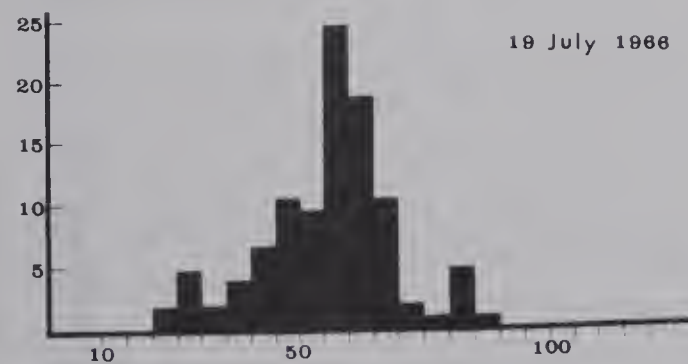
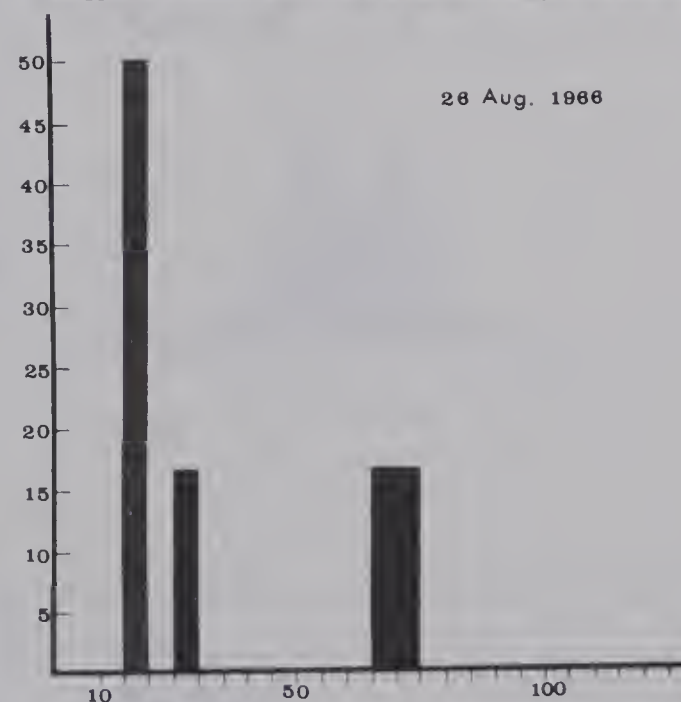
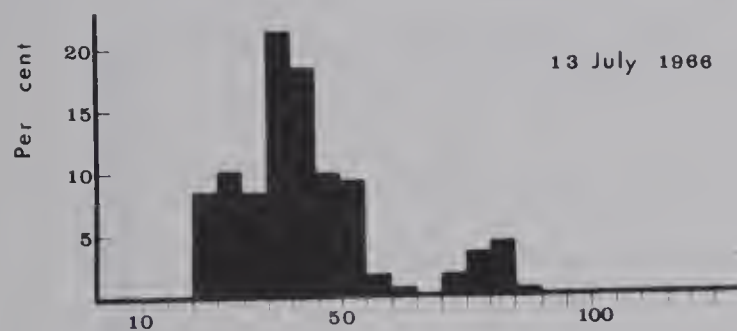
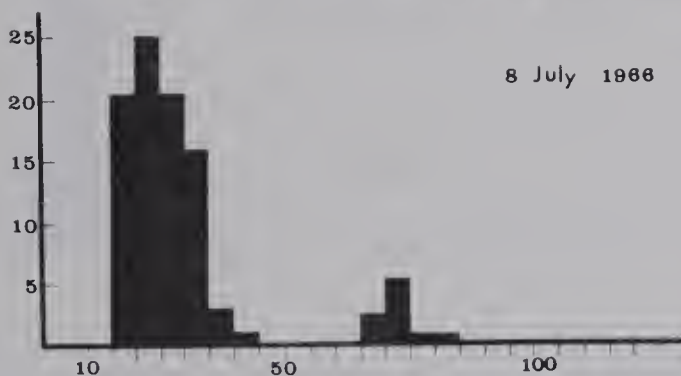
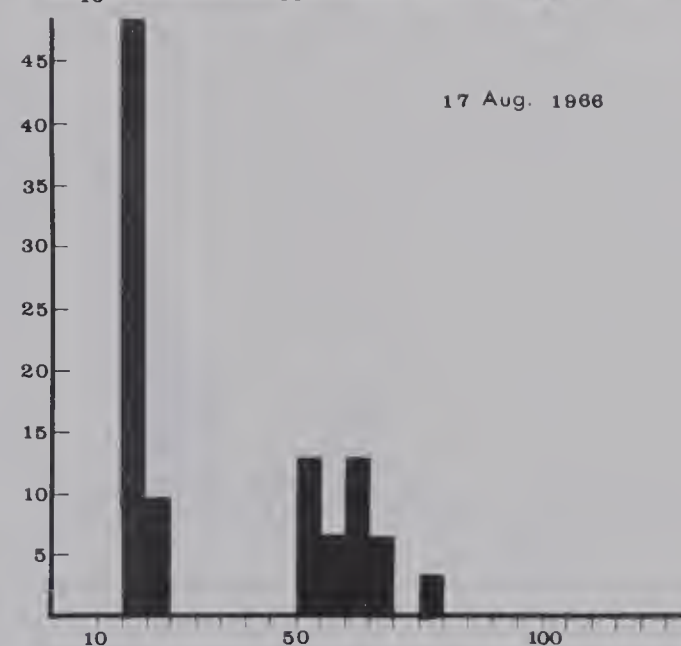
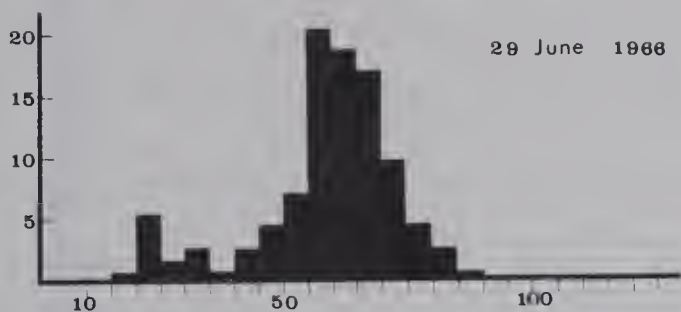
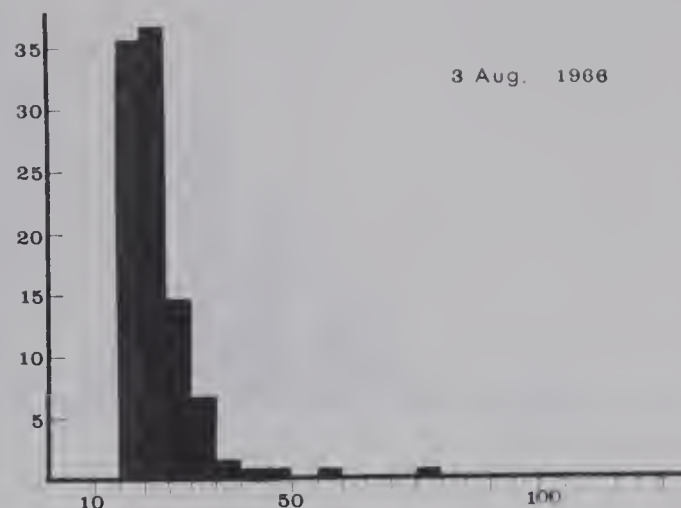
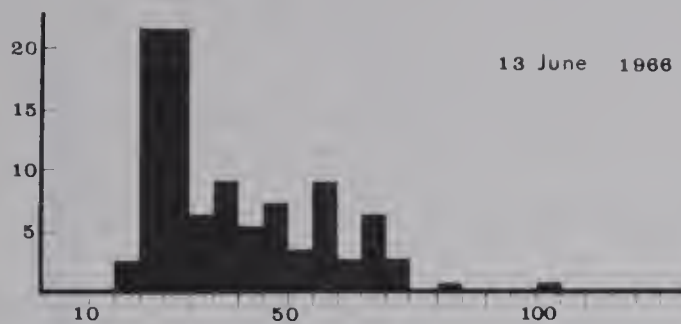
At least 100 females, except in samples containing fewer than 100 animals, were measured from the top of the head to the base of the spine. The data were grouped in a histogram similar to that used by Edmondson (1955), with each bar representing five eyepiece micrometer units or 0.16 mm (Fig. 5 & 6).

To interpret age structure of D. schødleri in relation to size-frequency data it is necessary to introduce some laboratory results, which are reported on in detail in Part V. The size of the first instar females developing from parthenogenetic eggs varied from 14.0 (0.46 mm) to 24.1 micrometer units (0.78 mm) with an average of 18.5 units (0.60 mm). The size of first adult instar of parthenogenetic females varied from 44.3 micrometer units (1.44 mm) to 70.0 units (2.28 mm) with an average of 54.7 units (1.78 mm). In the field study, the size of the smallest parthenogenetic female carrying eggs in the brood pouch was 50.0 units (1.63 mm), while the size of the smallest ehippial female was 54.5 units (1.77 mm). Therefore, individuals larger than 55 units were considered adults, and those 55 units or smaller were considered immature animals.

On 13 June 1966, the entire population was exclusively parthenogenetic and contained a larger proportion (77.3%) of immature females (Figs. 5 & 6). Most of the immature females belonged to the 20-30 unit group, the largest individual being in the 100-105 unit group. Slobodkin (1954) has shown that in the early stages of population growth the few adult animals will have a high reproductive rate, resulting in a size-frequency distribution that is skewed towards the small end. For D. schødleri, this is apparently the situation on 13 June, resulting in a rapidly reproducing population containing a larger proportion of small



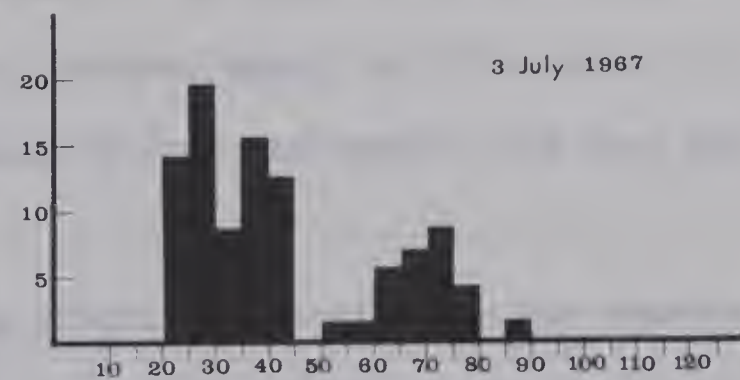
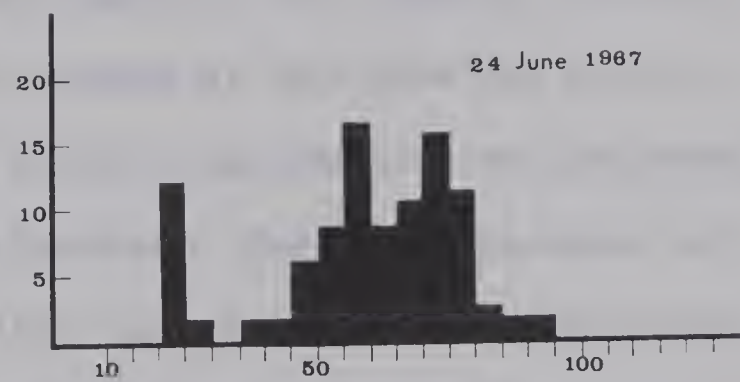
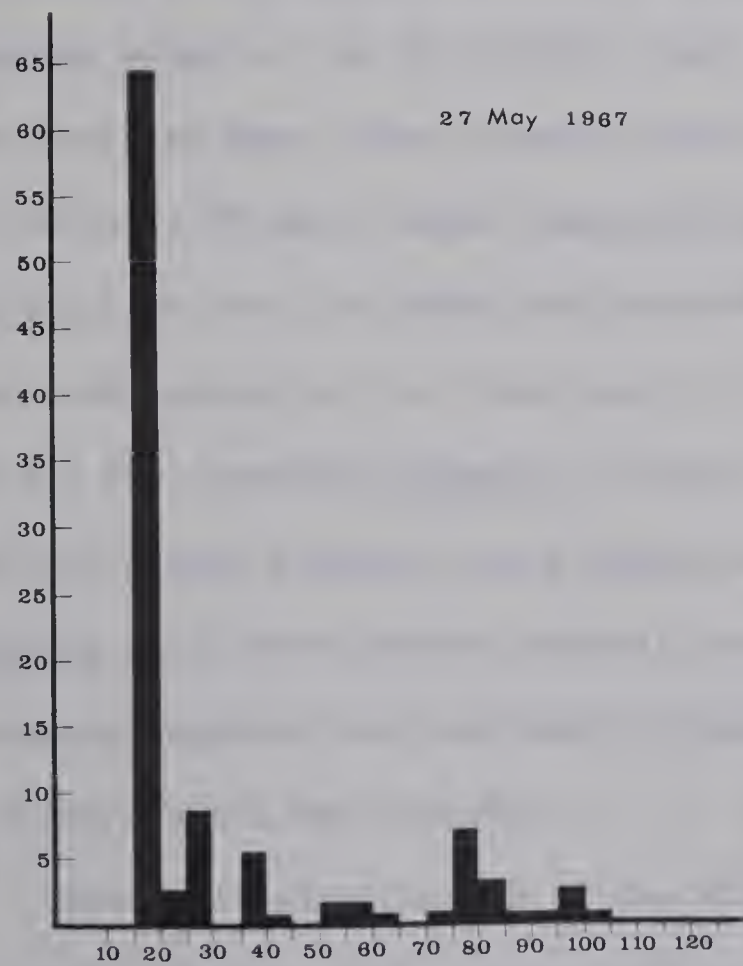
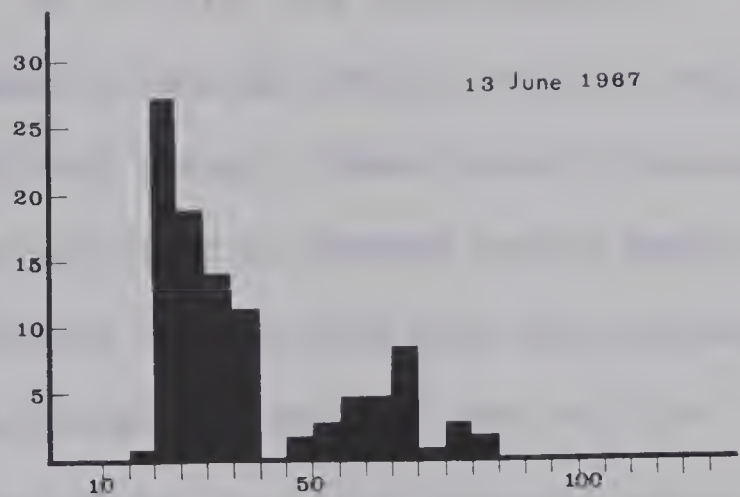
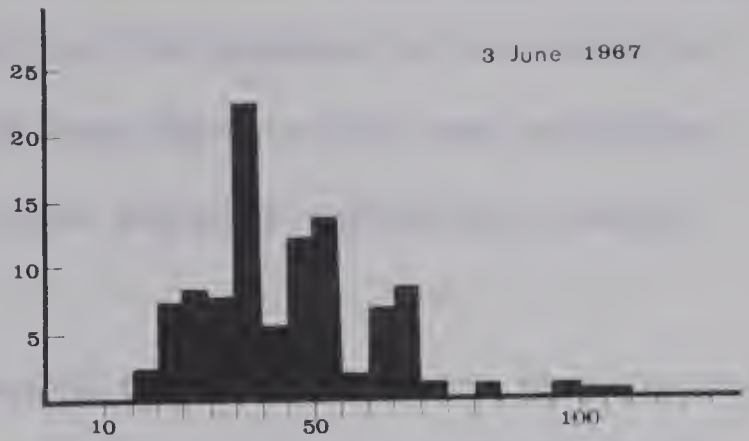
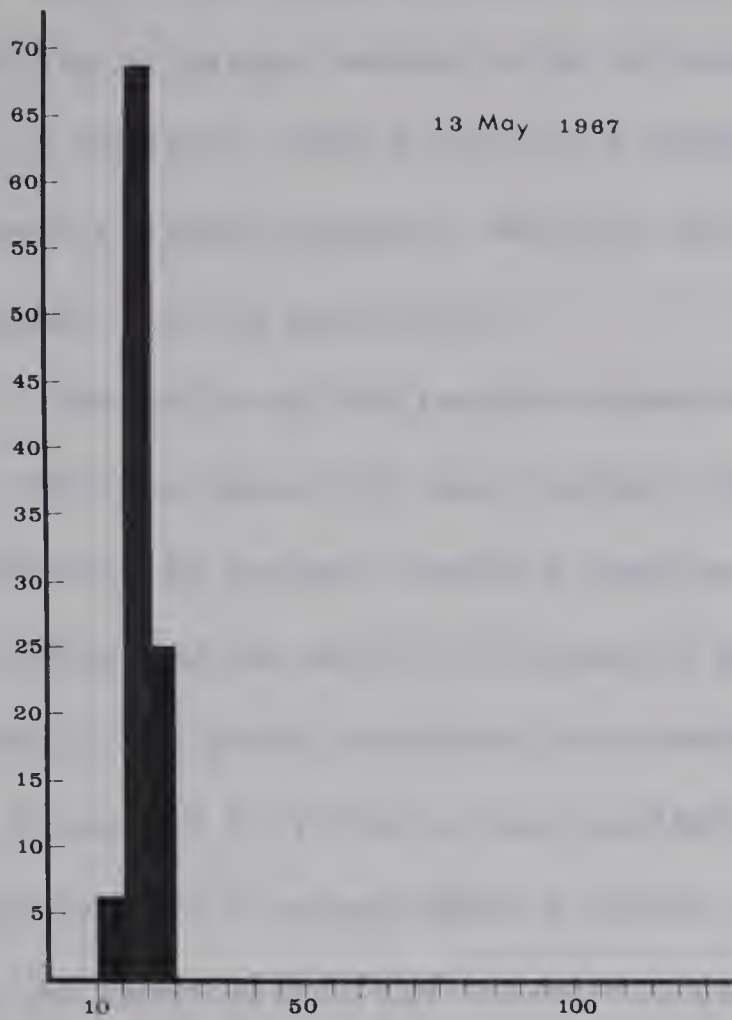
Figure 5. Population length-frequency histograms of Daphnia schødleri in Big Island Lake, 13 June 1966 to 16 October 1967. The histograms represent both non-ephippial and ehippial females, excluding male animals. Each individual histogram shows the percentage size distribution of the species of the indicated date. The width of each bar represents 5 micrometer units, or 0.16 mm.



Body length in micrometer units

Figure 6. Population length-frequency histograms of Daphnia schødleri in Big Island Lake, 13 May 1967 to 3 July 1967.

Per cent



Body length in micrometer units





animals. On 29 June, the size-frequency distribution of the population had shifted to larger animals with 74 percent of the population consisting of adult females. This is due to a reduced reproductive rate and continuous growth of small animals. Also at this time sexually reproducing females appeared in the population.

Mortality of the larger animals caused the size-frequency distribution to shift in favour of small animals again, and by 8 July, the population contained 88 percent immature females. On 13 July, the size-frequency distribution was still in favour of immature females (86.1%), but the small animals had grown to medium size range (35-45 units). These animals continued to grow, and by 19 July, the population contained 61 percent mature females, resulting in a second peak of larger animals. After this date and continuing to the remainder of the ice-free season there is a tendency for the size-frequency of the population to be discontinuous, but to be skewed towards smaller animals. On 16 October, very few daphnids were sampled, and none was carrying eggs. The largest animals sampled at this time had a size of 60 units (1.95 mm). Water temperature at this time was 4°C, and the surface of the lake near the shore had started freezing. The low temperature and low food content of the lake water at this time resulted in a slow maturity of the few remaining Daphnia, so that even the largest Daphnia collected (60 units) was probably not a mature animal. In warmer water this size Daphnia would have reached maturity and produced eggs. By 27 November, free swimming daphnids had completely disappeared from the sample, and they were not found until next spring.

The oscillatory nature of the size-frequency distribution was similar in 1967. The May 1967 samples indicate a rapidly growing population. On 3 June the size frequency distribution had shifted towards medium sized animals, but the proportion of immature females (79%) was still greater



than mature females. The reproductive rate, 13 eggs per clutch, was high at this time, maintaining a distribution favouring small animals. This distribution continued through 13 June. However, on this date ehippial females outnumbered parthenogenetic females indicating that the peak of reproduction had passed. This coupled with continuous growth of small animals resulted in the shift to larger animals on 24 June. A second size-frequency oscillation, similar to 1966, apparently began on 3 July 1967.

The larger animals, with sizes beyond 100 micrometer units (3.25 mm), were found only in the early summer months of both years (13 June 1966; 27 May and 3 June 1967), a time when water temperatures were low. From 27 July until 26 August 1966, when the water temperatures were higher, the largest animals sampled were never larger than 80 micrometer units (2.60 mm). MacArthur and Baillie (1929) have shown that the mean length of life for Daphnia magna Straus varied inversely with temperature, and that females cultured at lower temperatures reached a larger final size than those cultured at higher temperatures. This might explain the situation for D. schødleri in Big Island Lake, although the food supply might also be important.

#### Cyclic Reproduction in D. schødleri

##### Seasonal variation in egg production

Berg (1934), studying cyclic reproduction of Daphnia, believes that (1) a state of depression, produced by unfavorable external conditions, in Daphnia females kept in culture causes a change from parthenogenetic to gamogenetic reproduction, and (2) gamogenesis in nature is accompanied by a state of depression in the population in question. This supports the correctness of the hypothesis that, in nature, the transition from parthenogenesis to gamogenesis is caused by the influence of unfavourable





external conditions. These conditions cause states of depression in the females and thereby change their mode of reproduction and the mechanism of sex determination. Daphnia in cultures react to favourable conditions by producing large, parthenogenetic broods; they respond to unfavourable conditions (such as overcrowding, insufficient food, low temperatures, impurities in water, etc.) by producing smaller broods. The size of the parthenogenetic brood, therefore, may serve as a visible measure of the influence of life conditions.

To determine possible changes in brood size during the periods of parthenogenetic and gamogenetic reproduction in nature, the average number of parthenogenetic eggs was determined in a series of D. schødleri populations at different seasons, both when the population was in gamogenesis and in pure parthenogenesis. In 1966, the first quantitative sample was collected on 13 June. The mean egg number (i.e. mean clutch size) carried by mature females in this sample was 8.61, the entire population consisting of non-ephippial females (Table 4). On 22 June, the mean egg number had dropped to 5.87. At this time, many ehippial females and unattached ehippia were found in the sample. By 29 June, there were only two to four eggs in the brood pouch of each parthenogenetic (non-ephippial) female, and the mean egg number of mature females was only 2.5. There were also numerous ehippial females found in the population. Mean egg numbers oscillated during July and August, increasing to a value of 11 on 26 August (from 3 August until 26 August, the population was exclusively parthenogenetic). By 28 September the mean egg number had again declined, and at this time a few ehippial females were observed in the sample. On 16 October, a few immature females and males were collected; but neither ehippial females nor females carrying eggs or embryos were found in the sample. By 27 November free swimming Daphnia





Table 4. Seasonal variation in parthenogenetic egg production and the size of females carrying eggs.

Date	Mean egg number in brood pouch	Mean length of females with eggs mm	Water temperature C
13 June (1966)	8.61	2.32	-
22 June	5.87	2.70	-
29 June	2.50	2.60	23.0
8 July	5.40	2.56	24.3
13 July	7.20	2.64	20.9
19 July	3.90	2.07	22.5
27 July	4.60	2.04	19.5
3 Aug.	6.80	2.03	22.9
17 Aug.	7.40	2.17	16.9
26 Aug.	11.00	2.15	19.0
28 Sept.	3.90	2.32	6.2
27 May (1967)	29.90	2.62	14.8
3 June	13.10	2.30	14.7
13 June	6.70	2.63	14.5
24 June	6.30	2.50	19.0
3 July	4.20	2.57	18.7



had completely disappeared from the sample.

During 1967, the first immature free swimming Daphnia was encountered on 3 May; and only immature females were collected on 13 May. By 27 May, there were numerous parthenogenetic adult females, and the mean egg number was 29.9, the highest value for the entire study period. Thereafter mean egg numbers declined rapidly. By 13 June, the population of Daphnia had reached its peak, and the mean egg number had fallen to 6.7. At this time the production of ephippia was taking place (see Table 3). On 3 July, the mean egg number had decreased to a value of 4.2.

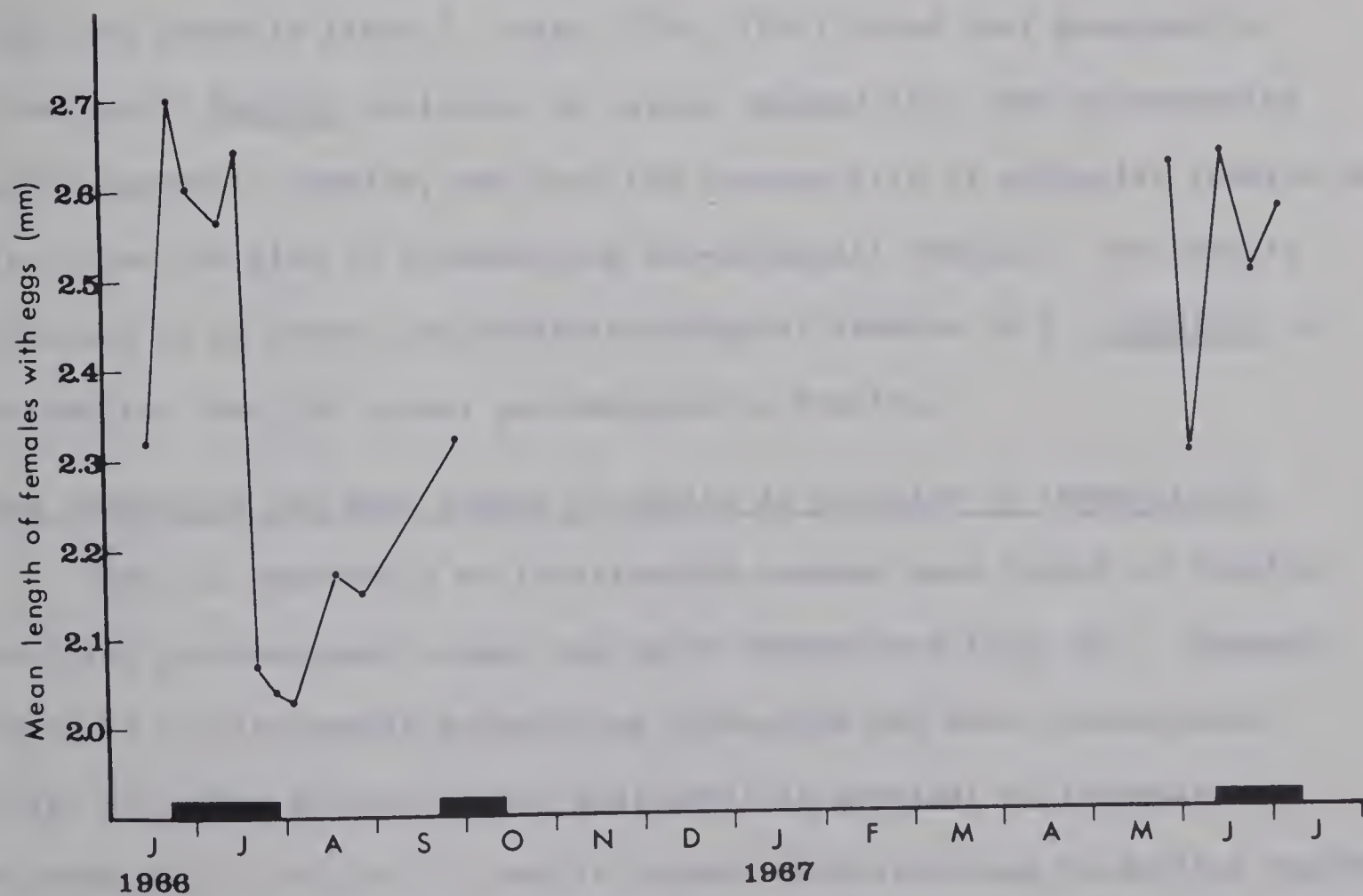
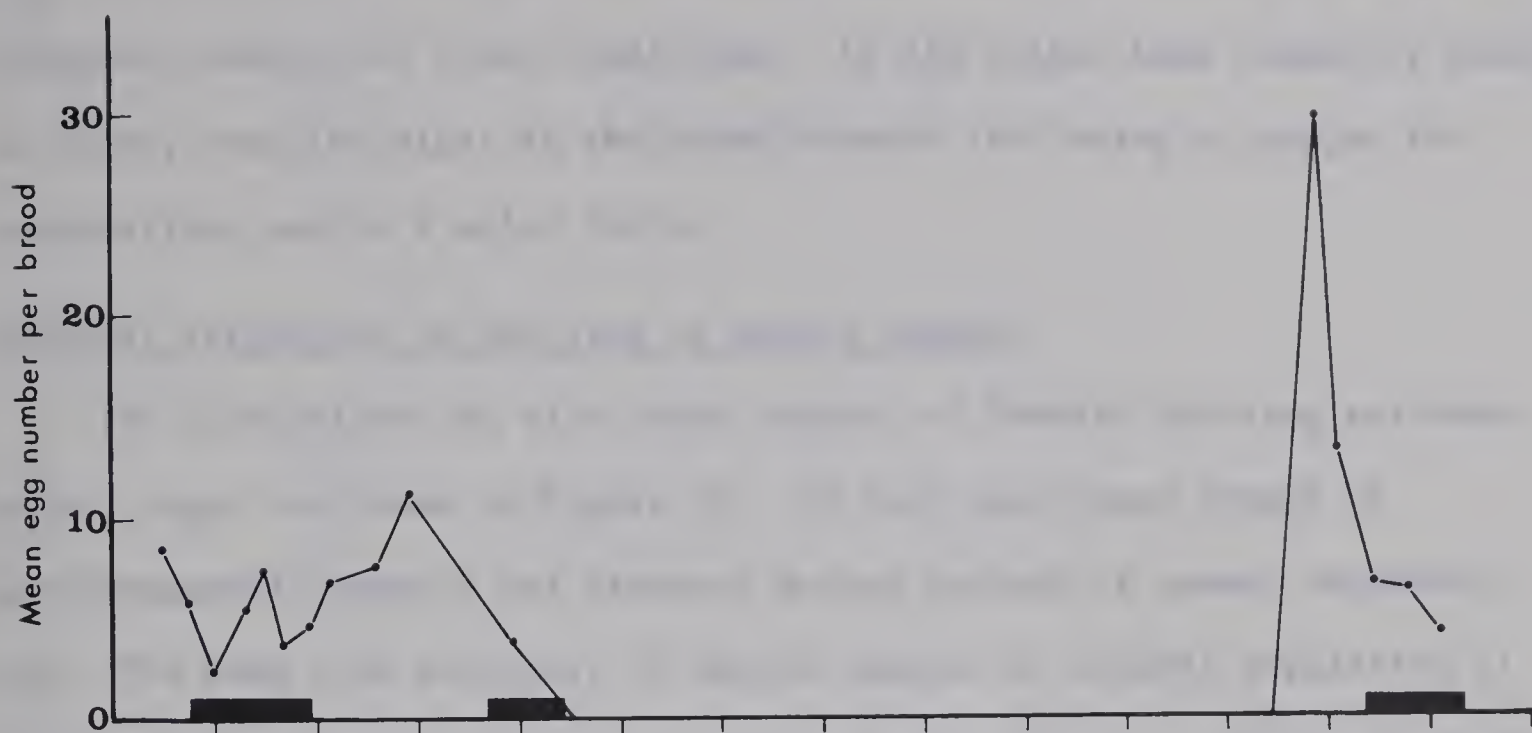
The average number of parthenogenetic eggs per female diminished immediately before the beginning of the sexual periods (Fig. 7a). This decrease in brood size was not due to a decrease in the average size of the egg-bearing females (Table 4). These observations are similar to those of Berg (1931, 1934) and support his hypothesis that a state of depression in parthenogenetic female Daphnia results in or is accompanied by sexual reproduction.

Degenerate eggs were frequently observed in the brood chamber of D. schødleri from Big Island Lake. These eggs were dark gray-brown and often appeared disintegrating. Normal eggs appeared blue-green or yellow-brown and were of a firm, less fragile texture. Degenerated eggs were excluded from the calculation of average brood size and no attempt was made to calculate the percentage of degenerate eggs in relation to total eggs present. On 22 June 1966 and 13 June 1967, when algae bloom of Microcystis and Anabaena were extensive, a large proportion of parthenogenetic females (about 20% of all mature females) were carrying disintegrating stage 8 embryos. These embryos were found clinging to Microcystis and Anabaena in the brood chamber of Daphnia. Brooks (1946) suggests that egg degeneration in Daphnia is caused by inadequate nutrition. Hall (1964), however,

Figure 7a. Seasonal variation in egg production of Daphnia schødleri in Big Island Lake. The periods when ehippial females occurred are indicated by black bars.

Figure 7b. Seasonal variation in the body length of mature parthenogenetic females of Daphnia schødleri in Big Island Lake. The periods when ehippial females occurred are indicated by black bars.







believes the causes are not so clear-cut; he suggests that degenerate eggs may reflect a specific nutritional deficiency, a change in food level, temporary anoxia, or other conditions. In Big Island Lake temporary anoxia at night, when the algae in the brood chamber are taking up oxygen for respiration, may be a major factor.

#### Seasonal variations in the size of mature females

The fluctuations in size (mean length) of females carrying parthenogenetic eggs are shown in Figure 7b. In both years mean length of parthenogenetic females was greatest during periods of sexual reproduction. The mean size structure of mature females in natural population is affected by the number of newly recruited mature females. During periods of sexual reproduction fewer newly matured females will be recruited, and hence the mean length of parthenogenetic females will be greater.

Seasonal variations in the mean length of females carrying resting eggs are shown in Table 5. Berg (1934, 1936) noted that gamogenetic (ephippial) Daphnia collected in nature weighed less than accompanying parthenogenetic females, and that the average size of ephippial females was less than the size of accompanying non-ephippial females. The results obtained in my study also indicate ephippial females of D. schødleri to be smaller than the normal parthenogenetic females.

#### Egg production and mean length of adults in relation to temperatures

There is apparently no relationship between mean length of females carrying parthenogenetic eggs and water temperature (Fig. 8a). However, there is a relationship between egg production and water temperature (Fig. 8b). Egg production per individual is greatest at temperatures between 14.5 C and 14.8 C; and at higher temperatures egg production declined sharply. From these data it appears that the optimum temperature for egg production in Big Island Lake is somewhere between 14.5 and 15 C.



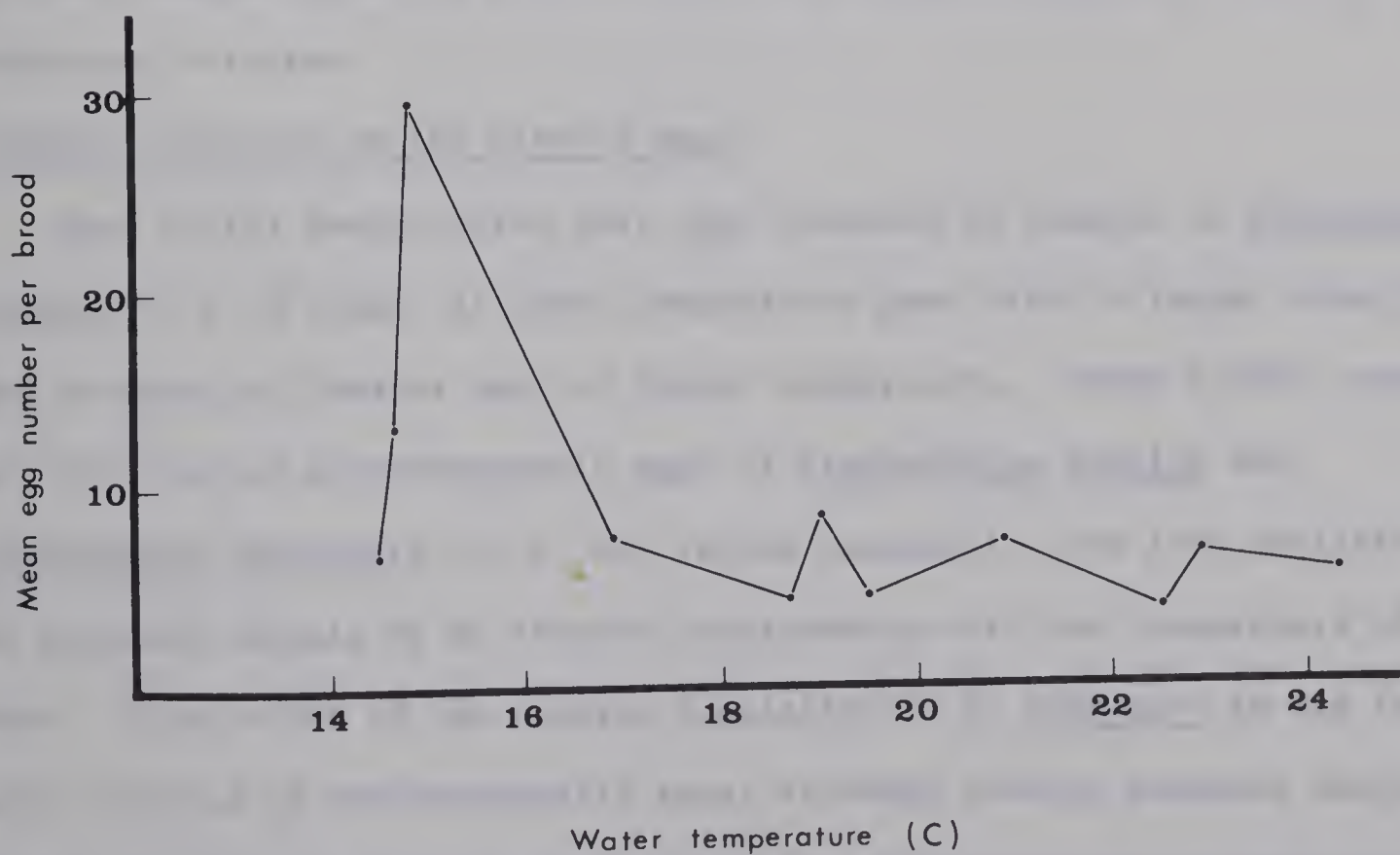
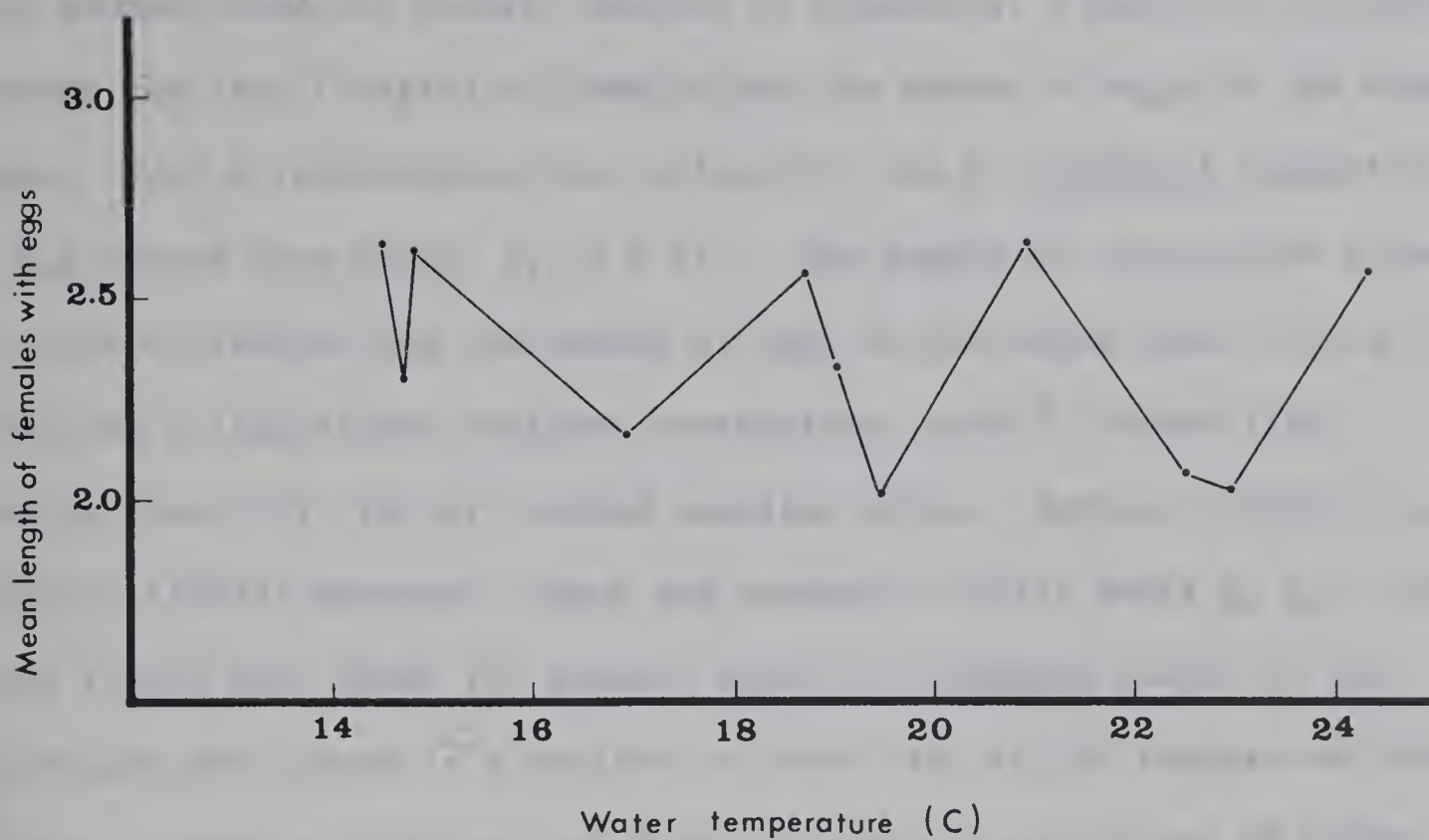
Table 5. Seasonal variation in the size of resting eggs and the gamogenetic (ephippial) females carrying these eggs.

Date	Mean length of gamogenetic females mm	Mean volume of resting eggs mm <sup>3</sup> x 10 <sup>3</sup>	Number of eggs measured	Water temperature C
1966				
29 June	2.14	6.98	32	23
8 July	2.29	9.45	43	24.3
13 July	2.28	8.21	32	20.9
19 July	1.83	4.23	4	22.5
27 July	2.26	4.03	2	19.5
1967				
13 June	2.20	8.07	76	14.5
24 June	2.38	8.12	51	19
3 July	2.29	7.20	35	18.7



Figure 8a. The relationship between temperature and size of mature parthenogenetic females of Daphnia schødleri in Big Island Lake.

Figure 8b. The relationship between temperature and parthenogenetic egg production of Daphnia schødleri in Big Island Lake.





### Egg production in relation to body size of mature females

Several workers (e.g. Green, 1954, 1955, 1956, 1966; Burgis, 1967) have demonstrated, for several species of Cladocera, a positive correlation between the size (length) of females and the number of eggs in the brood pouch. Such a relationship also exists for the D. schødleri population of Big Island Lake (Figs. 9, 10 & 11). The degree of correlation between the size of females and the number of eggs in the brood pouch (Table 6) indicates a significant positive correlation, with P (Probability) smaller than 0.01, for all tested sampling dates. Several workers: e.g. Kerherve (1927); Anderson, Lumer and Zupancic (1937); Banta et al. (1939); Green (1954) have shown for several species of Daphnia reared in the laboratory that there is a decline in brood size in the largest and oldest females. However, this was not found in natural populations of either Daphnia (Green, 1955) or Ceriodaphnia (Burgis, 1967). And it was not found in natural population of D. schødleri in Big Island Lake, suggesting that females probably do not survive as long in nature as they do in laboratory cultures.

### Seasonal variation in the size of eggs

Agar (1913) demonstrated that eggs produced by females of Simocephalus vetulus (O. F. M.) kept at lower temperature gave rise to larger young than eggs produced by females kept at higher temperature. Green (1966) reported that the size of parthenogenetic eggs of Simocephalus vetulus and Scapholeberis mucronata (O. F. M.) varied seasonally, and this variation was governed largely by an inverse relationship with the temperature of water. In my study of the natural population of D. schødleri in Big Island Lake, the size of parthenogenetic eggs, although showing seasonal variations (cf. Table 7), did not show a definite relationship with temperature.

Figure 9. The relationship between egg production (egg number per brood) and size of mature parthenogenetic females of Daphnia schødleri in Big Island Lake for samples collected on 13 July, 19 July and 27 July 1966.



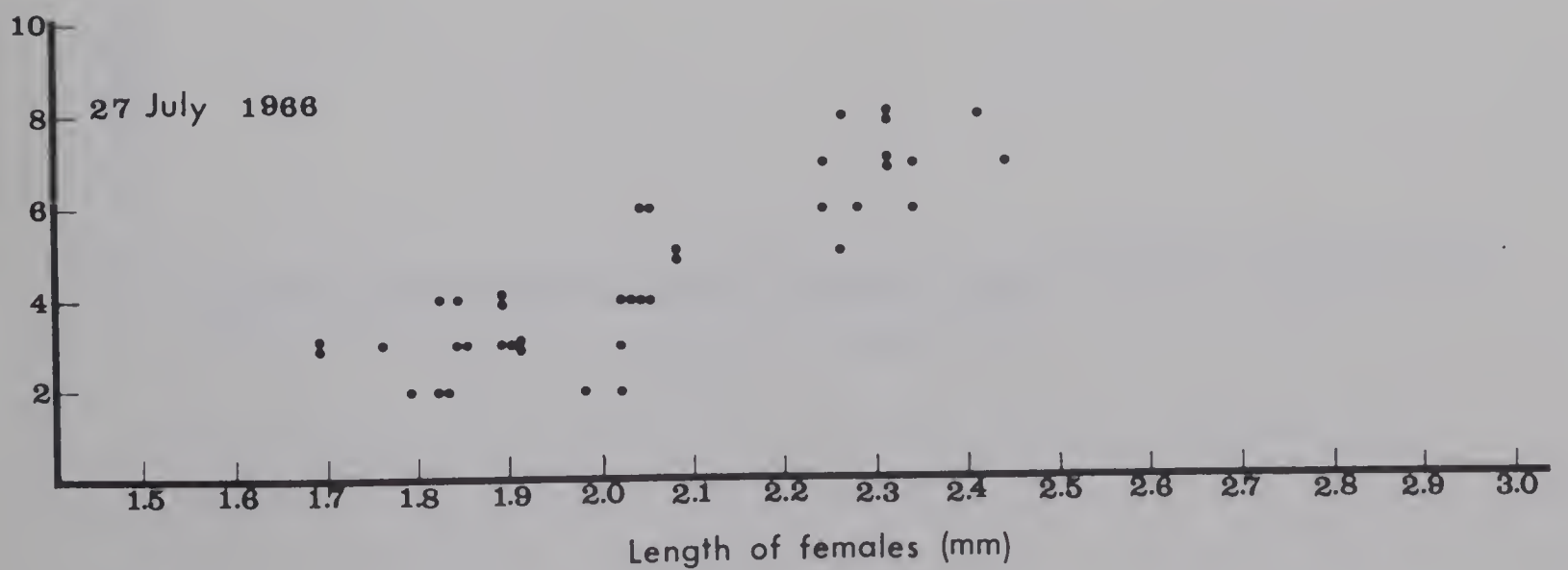
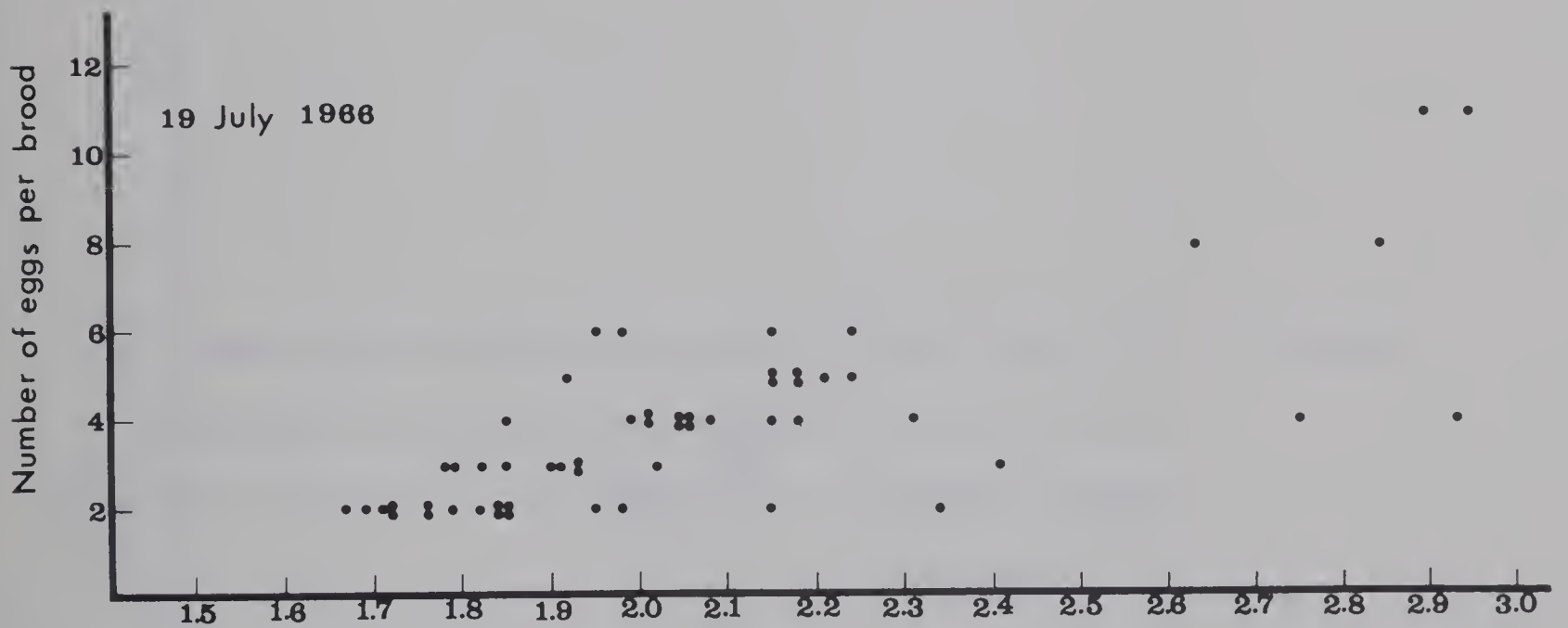
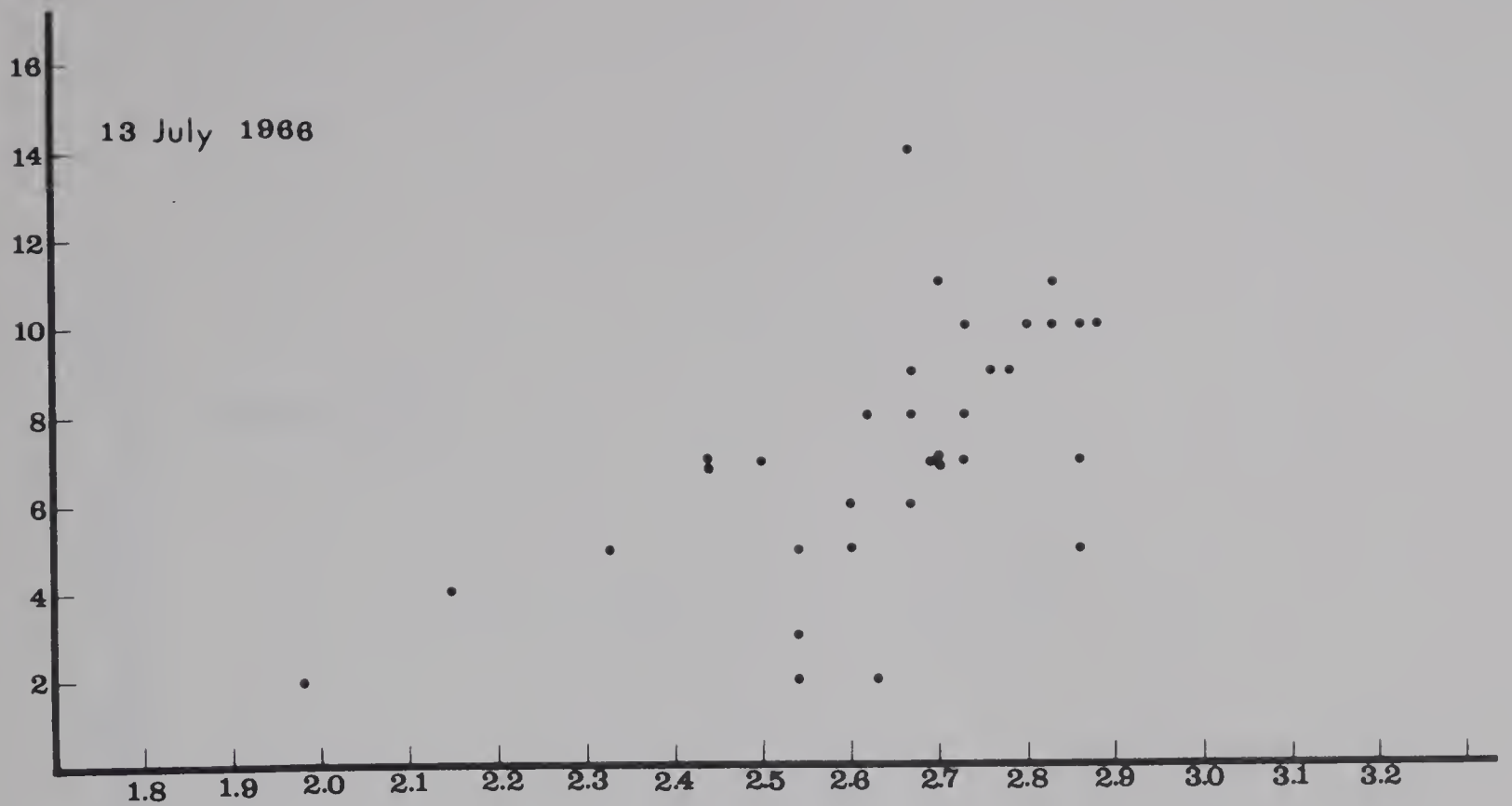


Figure 10. The relationship between egg production (egg number per brood) and size of mature parthenogenetic females of Daphnia schødleri in Big Island Lake for samples collected on 27 May 1967.

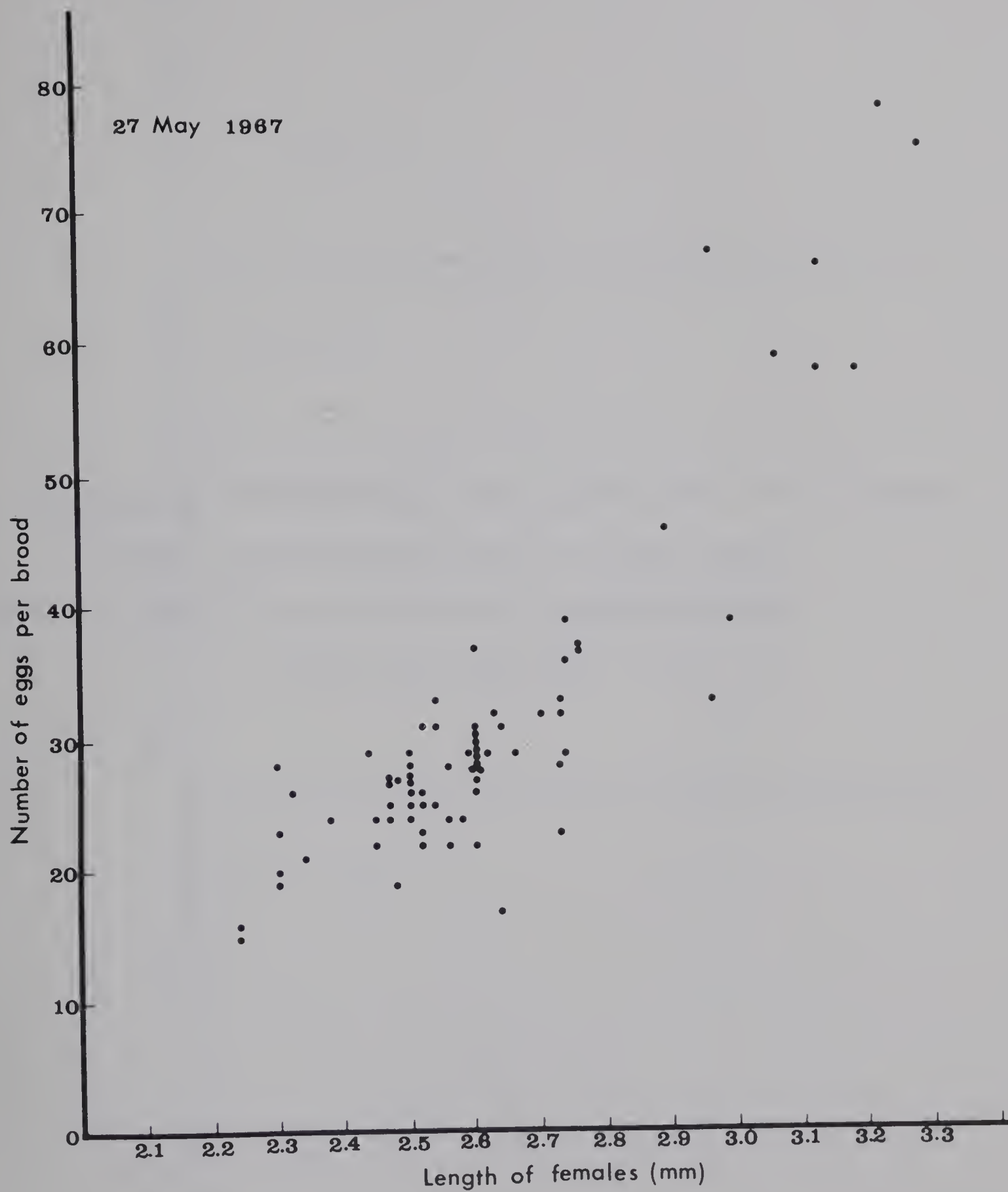


Figure 11. The relationship between egg production (egg number per brood) and size of mature parthenogenetic females of Daphnia schødleri in Big Island Lake for samples collected on 3 June, 13 June and 24 June 1967.

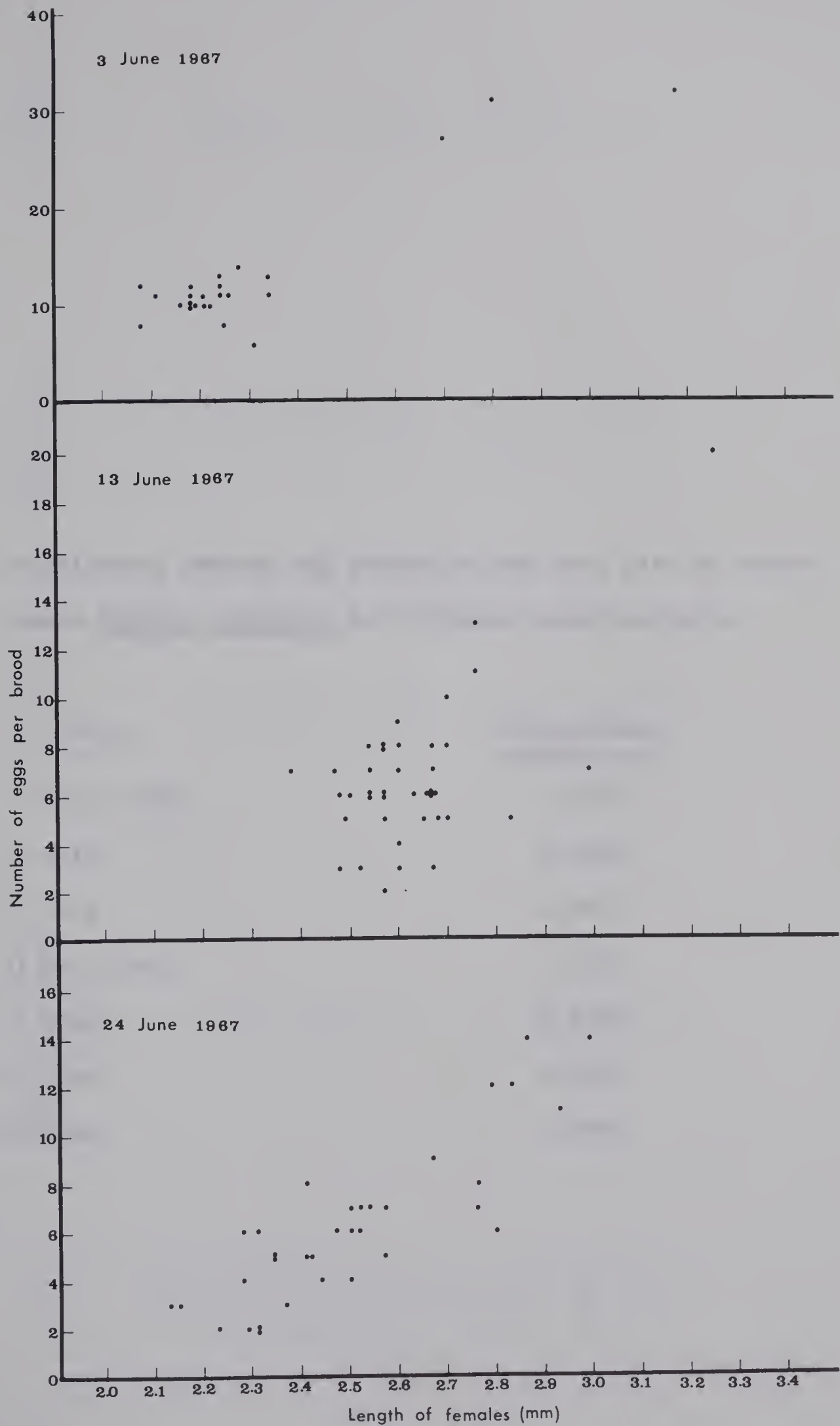






Table 6. Relationship between egg production and body size of mature female Daphnia schødleri for different sampling dates.

Date	Correlation coefficient
13 July (1966)	0.6023
19 July	0.7402
27 July	0.8651
27 May (1967)	0.9155
3 June	0.6103
13 June	0.8522
24 June	0.9340



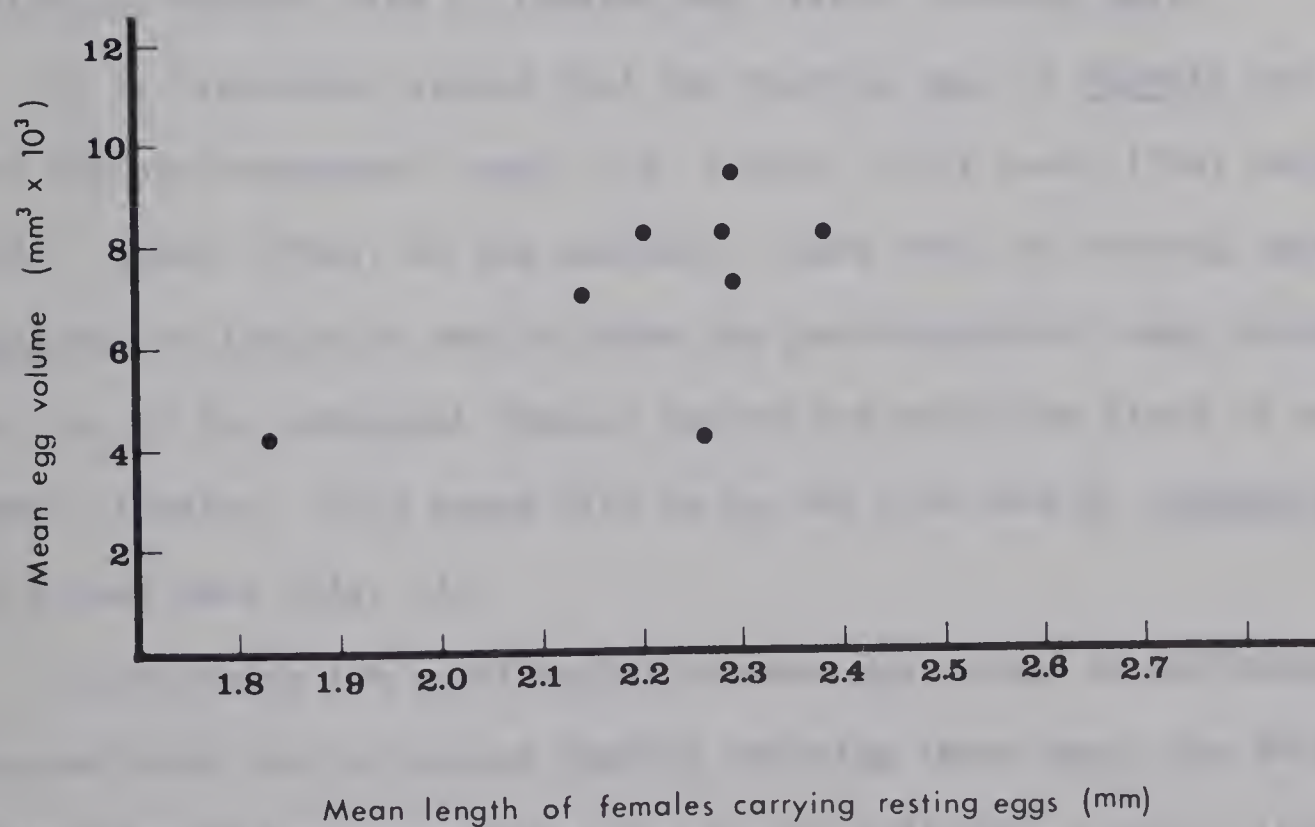
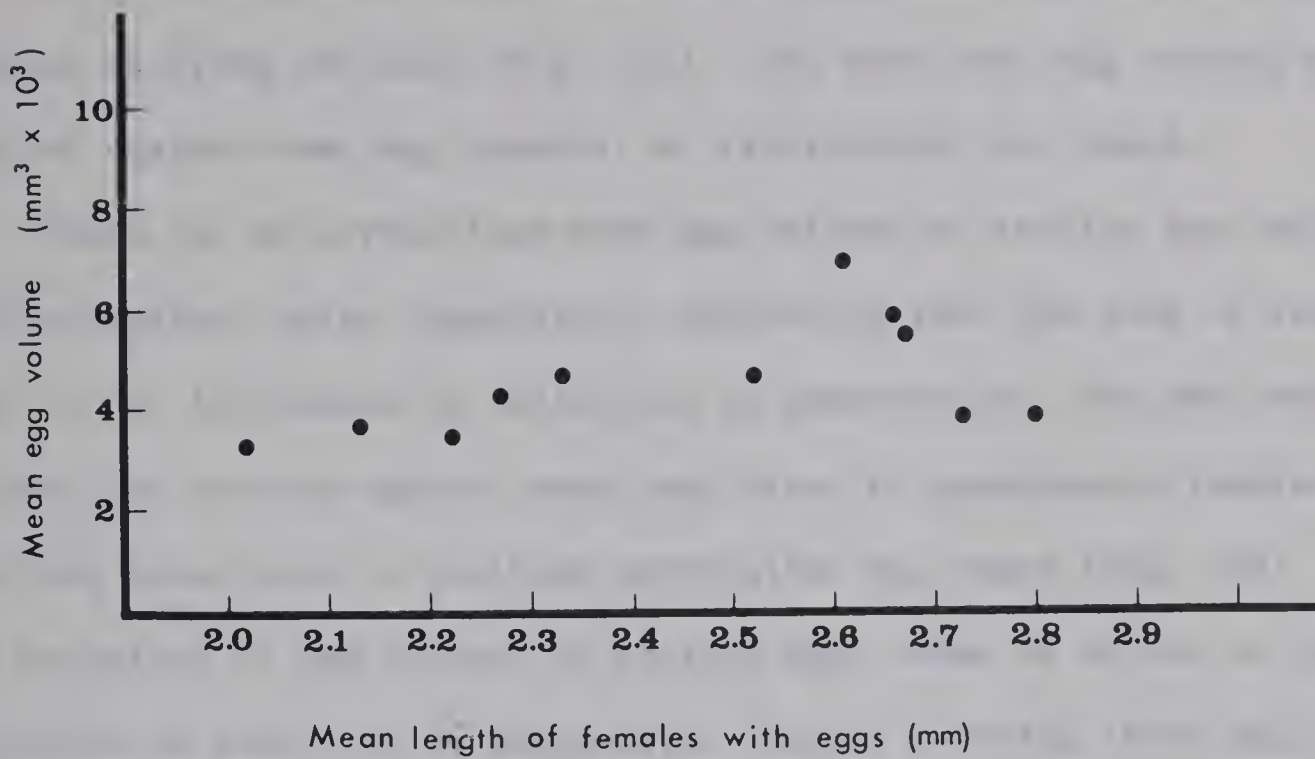
Table 7. Seasonal variation in the size of parthenogenetic eggs and females carrying these eggs.

Date	Mean length of parthenogenetic females mm	Mean egg volume mm <sup>3</sup> x 10 <sup>3</sup>	Number of eggs measured	Water temperature C
1966				
8 July	2.67	5.59	27	24.3
13 July	2.80	3.82	38	20.9
19 July	2.21	3.42	47	22.5
27 July	2.02	3.19	50	19.5
3 Aug.	2.13	3.53	31	22.9
17 Aug.	2.29	4.08	22	16.9
1967				
27 May	2.73	3.94	217	14.8
3 June	2.33	4.63	120	14.7
13 June	2.66	5.96	3	14.5
24 June	2.61	6.88	49	19
3 July	2.53	4.70	21	18.7

Figure 12a. The relationship between size (egg volume) of parthenogenetic eggs and size of female Daphnia schødleri carrying these eggs, based on the data from Table 7.

Figure 12b. The relationship between size (egg volume) of resting eggs and size of female Daphnia schødleri carrying these eggs, based on data from Table 5.







There is a relationship between egg volumes (sizes) of parthenogenetic eggs and the sizes of the females carrying these eggs, i.e. the size of parthenogenetic eggs seems to be influenced by the body size of females carrying the eggs (Fig. 12a). But when mean egg volumes were plotted against mean egg numbers, no relationship was found.

There was no correlation when egg volumes of resting eggs were plotted against water temperature, indicating that the size of resting eggs is not influenced by variations in temperature. But when mean egg volumes was plotted against mean body sizes of gamogenetic females carrying these eggs, a positive correlation was found (Fig. 12b). Hence the variation of egg volumes in resting eggs seems to be due to the variation in body size of gamogenetic females carrying these eggs.

#### Egg volume in relation to body size of mature females

The egg size of resting eggs was positively correlated with the body size of females carrying these eggs (Fig. 13). The calculated correlation coefficient was 0.7537 ( $P < 0.01$ ), indicating a positive relationship between size of females and size of resting eggs.

It is frequently stated that the resting eggs of Daphnia are larger than the parthenogenetic eggs (e.g. Storch, 1925; Lack, 1954; Banta et al., 1939). Green (1956), on the contrary, found that the resting eggs of D. magna may be larger or smaller than the parthenogenetic eggs depending on the size of the ephippial females and on the nutritive state of parthenogenetic females. This seems also to be the case with D. schødleri of Big Island Lake (Fig. 13).

To determine the relationship between egg volume of parthenogenetic eggs and body size of mature females carrying these eggs, the data were plotted double logarithmically; the result indicates a nearly linear relationship (Fig. 14). The log-log regression of egg volume to body

Figure 13. The relationship between size of resting (ephippial) eggs and size of ephippial females, and between size of parthenogenetic eggs and parthenogenetic females. The data for resting eggs based on a sample of 104 fresh ephippial females of Daphnia schødleri collected on 24 June 1967. The data for parthenogenetic eggs based on 136 fresh eggs dissected out of parthenogenetic females collected on 24 June 1967. The dotted line indicates the mean volume of parthenogenetic eggs.

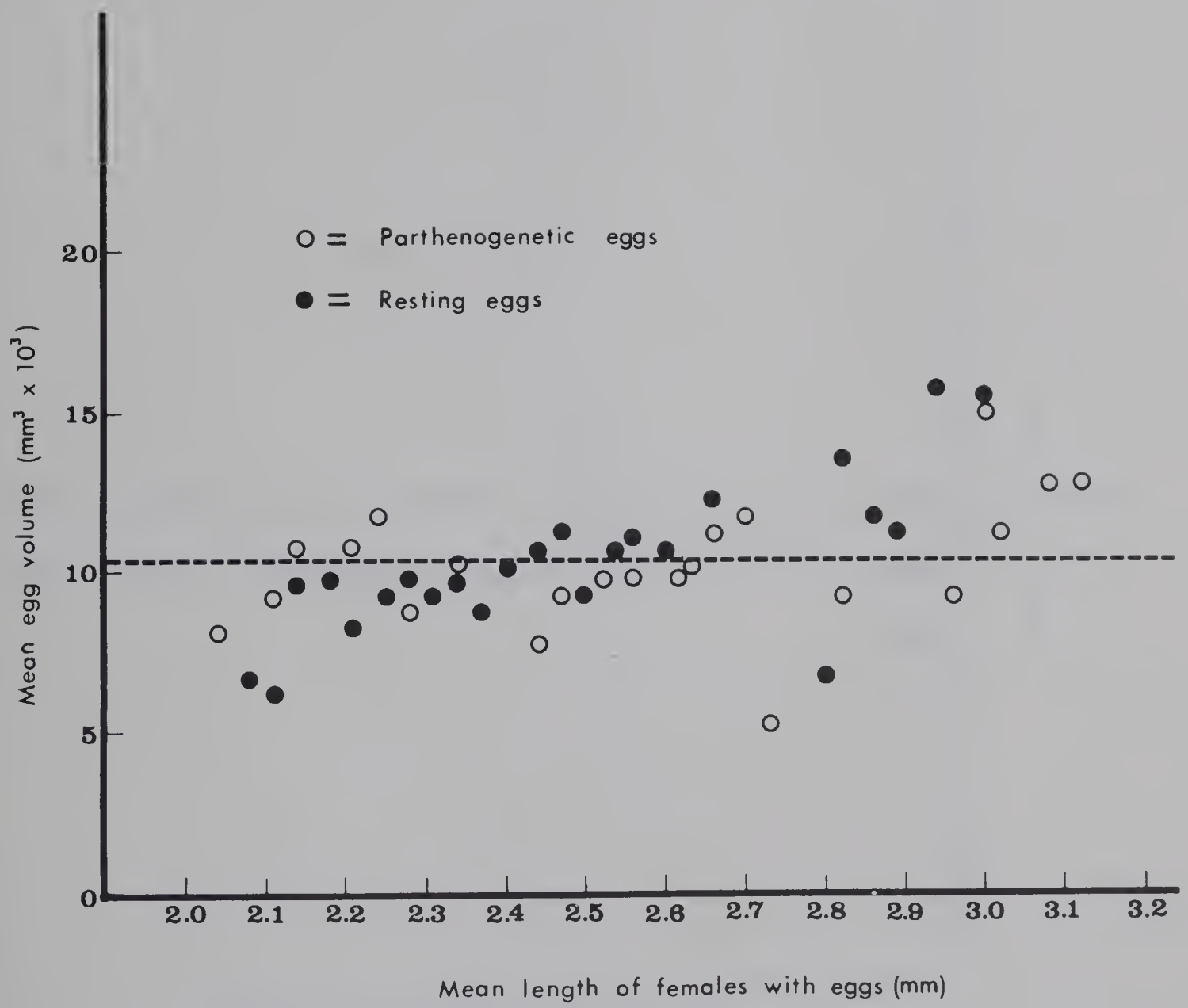
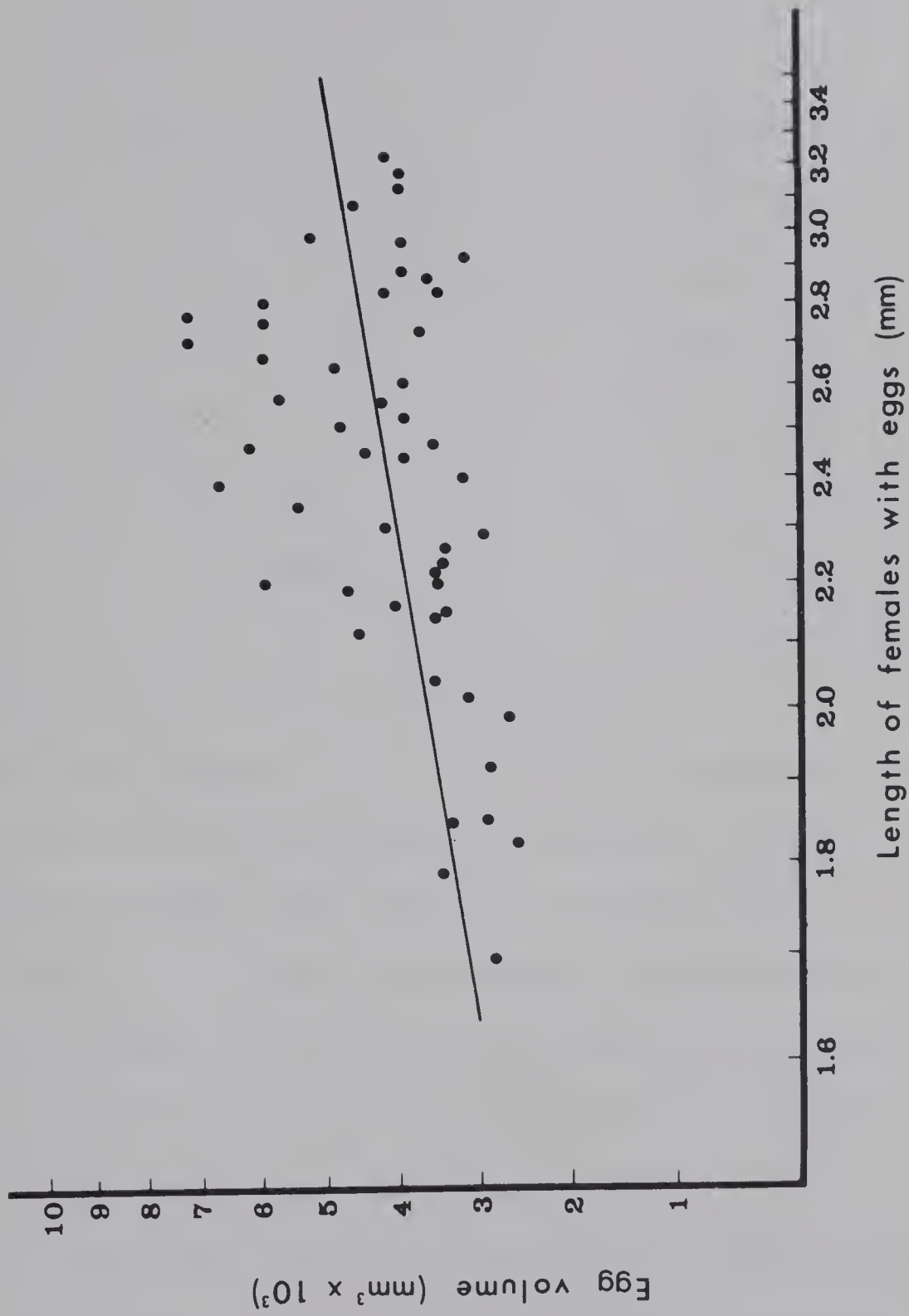




Figure 14. The log-log relationship between size of parthenogenetic eggs and size of female Daphnia schødleri carrying these eggs.





length was  $\log Y = 0.3424 + 0.6909 \log X$  (or  $Y = 2.20 X^{0.6909}$ ), where  $Y$  is the egg volume of parthenogenetic eggs in  $\text{mm}^3 \times 10^3$ , and  $X$  is the body length in millimeters of females carrying the eggs.





## LABORATORY STUDIES

Life History of Daphnia

The life history of Daphnia can be divided into four main periods, i.e. egg, juvenile, adolescent, and adult. The young daphnids after hatching from either resting or parthenogenetic eggs pass through several juvenile instars. The number of juvenile instars varies with the species, and even within species: e.g. 3 instars in Daphnia longispina (O. F. M.) (Banta et al., 1939); 3-4 in D. pulex Leydig emend. Richard (Anderson and Jenkins, 1942); 3-4 in D. schødleri (LeSuer, 1959); 4-5 in D. atkinsoni Baird (Green, 1956); 3-5 in D. thomsoni Sars (Green, 1956); 2-3 in D. obtusa Kurz emend. Scourfield (Green, 1956); and 3-4 in D. curvirostris Eylmann emend. Johnson (Green, 1956). There is only one adolescent instar for Daphnia, at which time the first clutch of eggs have matured in the ovary. The juvenile instars and adolescent instar collectively are called pre-adult instars.

When the female Daphnia molts at the end of adolescent instar, it enters the first adult instar, and the first clutch of eggs is promptly laid into the brood pouch. While the first clutch of eggs is developing in the brood pouch, the second clutch of eggs is being formed in the ovaries. Shortly before the end of the first adult instar, the fully developed young are released from the mother's brood pouch; the female Daphnia then molts and increases in size, and then the second clutch of eggs is laid into brood pouch. Thereafter, at the close of each adult instar, four events follow one another in rapid succession, usually within minutes or a few hours at most. They are (1) the release of young from the brood pouch to the outside, (2) molting, (3) increase in size, and (4) the depositing of a new clutch of eggs into the brood pouch. These



four events normally occur in each adult instar until near the end of the reproductive period.

The number of adult instars is much higher than that of pre-adult instars, varying with the species and with different conditions under which the animal lives. Banta et al. (1939) found up to 19 adult instars in D. longispina; Anderson and Jenkins (1942) found that D. magna females maturing in the fifth, sixth, and seventh instars had an average of 12.5, 12.2 and 12.3 adult instars respectively; LeSuer (1959) found up to 22 adult instars in D. schødleri; Elbourn (1966) found five to nine adult instars in D. obtusa.

Male Daphnia also pass through several juvenile instars and one adolescent instar before entering the adult instars. The adult male, as indicated by the presence of sperm in the testes, the shape of the ventral margin of carapace, and the size of the hook on the first thoracic leg, usually become sexually mature earlier than the female. In my study of D. schødleri, of the 23 males observed, 21 reached sexual maturity in the fourth instar and two in the fifth instar.

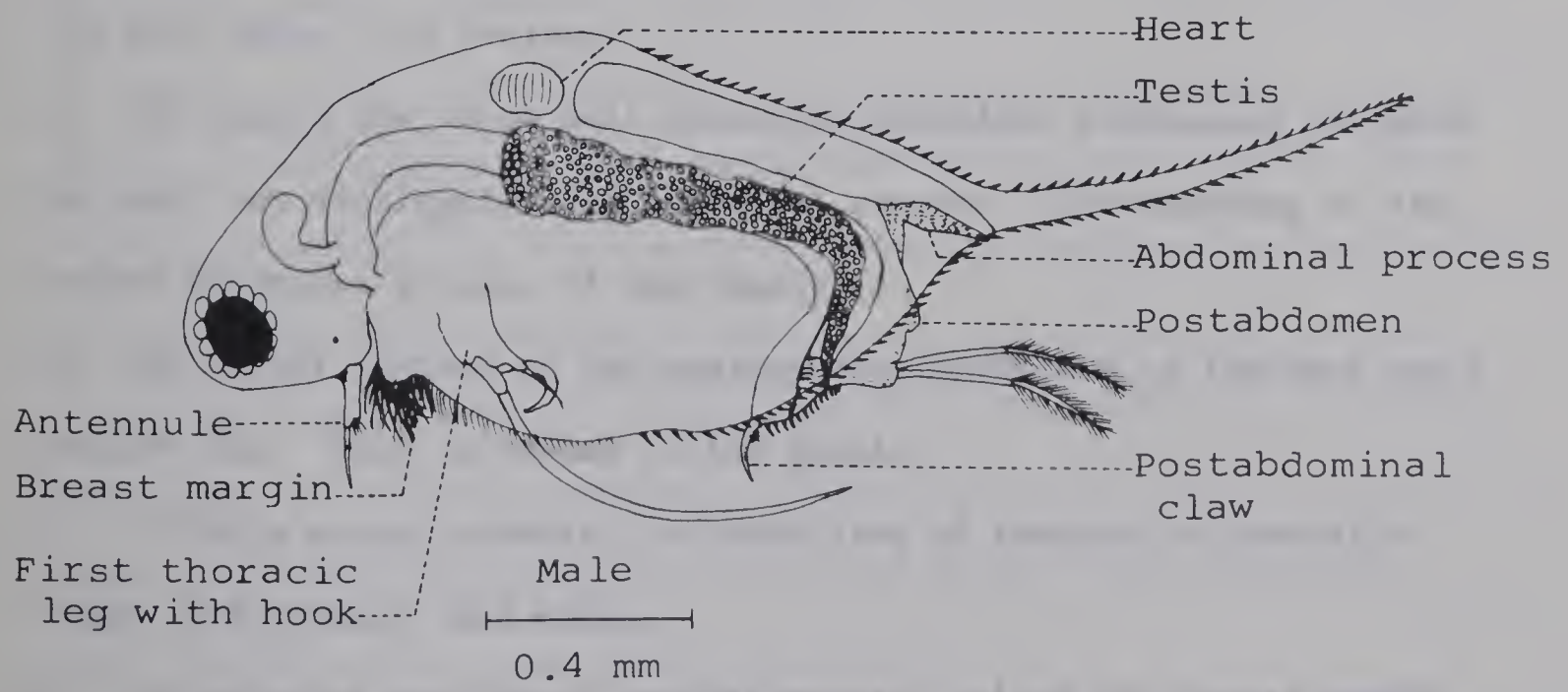
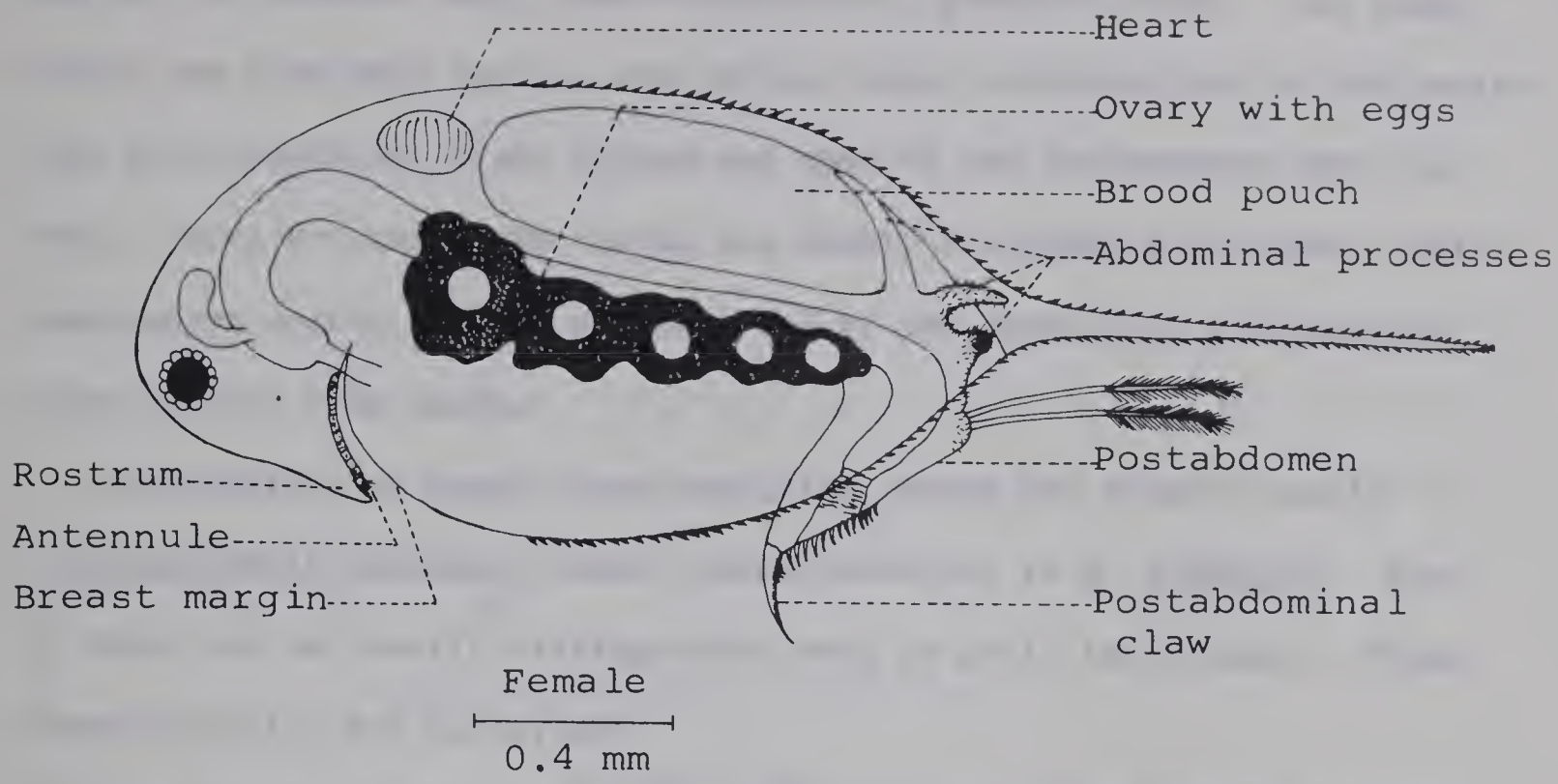
#### Sexual Dimorphism in D. schødleri

The sex of D. schødleri can be easily recognized both in immature and mature animals. The transparent character of the carapace and other body structures of Daphnia makes the internal organs clearly visible, and hence the presence of testes or ovaries, as well as the secondary sex characters can be observed in the living animal.

Figure 15 is an outline drawing of a female and male D. schødleri. Gonads of D. schødleri can be easily distinguished only in sexually mature animals. The two elongated ovaries lie lateral or somewhat ventral to the intestine in the thoracic region of the body. Depending upon the reproductive stage the ovaries may be densely granular with large oogonial nuclei, or they may contain a mass of large, irregularly granular yolky

Figure 15. The outline drawings of adult female and male Daphnia schødleri showing definitive and secondary sexual characteristics. In the male, the second antennae and all but the first thoracic legs are omitted; while in female, the second antennae and all thoracic legs are omitted.









eggs. The oviduct is located dorsal and at the posterior (caudal) end of the ovary. It leads into the brood pouch; but it is very delicate and cannot be seen except when egg laying is in progress. The male testes are smaller and contain small, semi-transparent, granular sperm. The sperm ducts, one from each testis, are narrow caudal continuations of the testes that pass laterally to the rectum and open on the postabdomen near the anus. In mature males the testes are widely distended with round, semi-transparent sperm, and the proximal end of the sperm duct also becomes quite dilated with sperm.

In addition to gonad characteristics, there are several easily distinguishable secondary sexual characteristics in D. schødleri. Most of these can be clearly distinguished only in adult individuals. These characteristics are as follow:

- 1) The female antennules are small, non-movable, and are placed behind the rostrum. Male antennules are large, movable, and with a long stout anterior seta or flagellum.
- 2) The head of the female possesses a well marked and pointed rostrum. The male lacks this rostrum.
- 3) The female has three well developed abdominal processes; the male has only one distinguishable abdominal process, corresponding to the second abdominal process of the female.
- 4) The dorsal surface of the postabdomen (abreptor) of the male has a shallow bay. This is absent in the female.
- 5) In fully mature animals, the body size of females is generally larger and broader than males.
- 6) The anterior portion of ventral margin, called the breast margin by Banta et al. (1939), of the female curves in a fairly uniform fashion from the region of the rostrum to the posterior portion of the ventral



margin, and there are no hair-like projections (or bristles) on it.

In mature male the breast margin is not uniformly rounded, but is somewhat angular and has a moderate excavation just posterior to the rostrum region; it also bears numerous long, hair-like bristles.

7) The first thoracic leg of the female is relatively slender and bears numerous slender, feathery filaments, and the leg is without a hook. In the male the first thoracic leg is heavier and the first filament is longer than that of the female, while the remaining filaments are shorter and more slender than in the female. The first thoracic leg of the male also bears a stout, somewhat transparent chitinized hook, which serves to clasp the females during copulation.

#### Numer of Pre-adult Instars in

##### D. schødleri

#### Temperature and pre-adult instar number

Thirty two female animals were observed at room temperatures fluctuating from 22 to 29.4 C. Two animals died, one in the fourth and one in the fifth instar, before reaching sexual maturity. Of the remaining 30 females, eight became primiparous (reached the first adult instar and produced parthenogenetic eggs) during the fifth instar, and 22 became primiparous during the sixth instar. Hence the number of pre-adult instars at temperatures between 22 and 29.4 C varied from four to five.

Of 85 females kept in a water bath with the temperature maintained at  $20 \pm 1$  C, 57 became primiparous during the fifth instar, 27 during the sixth instar, and one during the seventh instar. Thus the number of pre-adult instars varied from four to six at 20 C.

Twenty one females were cultured in a refrigerator with the temperature maintained at  $5 \pm 1$  C. Six daphnids became primiparous during the sixth instar, 10 during the seventh instar, and two during the eighth







Table 8. Length of young in first instar and instar in which they reached maturity, at  
20 ± 1 C.

Length in first instar mm	Number reared	Number mature in					
		5th instar		6th instar		7th instar	
		No.	%	No.	%	No.	%
0.4550-0.4875	1	-		1	100.0	-	
0.4875-0.5200	3	-		2	66.7	1	33.3
0.5200-0.5525	7	2	28.6	5	71.4	-	
0.5525-0.5850	12	8	66.7	4	33.3	-	
0.5850-0.6175	8	5	62.5	3	37.5	-	
0.6175-0.6500	30	22	73.3	8	26.7	-	
0.6500-0.6825	9	6	66.7	3	33.3	-	
0.6825-0.7150	8	7	87.5	1	12.5	-	
0.7150-0.7475	2	2	100.0	-		-	
0.7475-0.7800	1	1	100.0	-		-	
0.7800-0.8125	4	4	100.0	-		-	



instar. The three remaining animals reached sexual maturity during the seventh instar, but instead of producing parthenogenetic eggs these females produced ephippia and hence sexual eggs.

It is obvious that temperatures influence the number of pre-adult instars, i.e. low temperatures increases the number of pre-adult instars. It is likely low temperatures delay the sexual maturity of daphnids, and hence increase the number of pre-adult instars.

#### Size at first instar and pre-adult instar number

Eighty five female D. schødleri were raised individually from the first instar to the production of the first clutch of eggs. Each individual was cultured in a small specimen bottle with 20 ml of diluted Banta's manure-soil medium and kept in a water bath with temperature maintained at  $20 \pm 1$  C. The medium was changed each time the animal molted. The initial length of each animal and the number of instars passed before eggs were laid were then noted. The results are presented in Table 8. As initial size in the first instar increased, the percentage of animals becoming mature in the fifth instar increased, and the percentage of animals becoming mature in the sixth instar decreased. Therefore, the initial size of the young liberated from the brood chamber may influence the instar in which maturity is reached.

For several species of Daphnia, Green (1956) found that animals becoming sexually mature in an early instar usually had a larger initial size than animals becoming sexually mature in a later instar. Results of my study of D. schødleri support these observations (Table 9).

The number of pre-adult instars in D. schødleri, collected from Big Island Lake varied from four to seven. The results of other studies also indicate a varying number of pre-adult (pre-reproductive) instars, e.g. 4-8 in D. magna (Anderson, 1932; Anderson and Jenkins, 1942); 4-5 in D. magna (Green, 1954 and 1956); 4-5 in D. pulex (Anderson, Lumer and Zupancic,



Table 9. The initial size and the size in the first adult instar of female Daphnia schødleri.

Culture condition	Mean length in first instar mm	Number of animals	First clutch of eggs laid instar	Mean length in the first adult instar mm
Room temperatures, 22 to 29.4 C	0.5480	22	5th	1.6400
	0.5441	8	6th	1.7385
Water bath 20 ± 1 C	0.6404	57	5th	1.7975
	0.5866	27	6th	1.8048
	0.4875	1	7th	1.7550
Refrigerator, 5 ± 1 C	0.7001	6	6th	2.0751
	0.6188	10	7th	2.1993
	0.6435	3	7th*	1.9110
	0.6338	2	8th	2.3075

\* Denotes producing ephippia





1937; Green, 1956); 3-5 in D. obtusa (Green, 1956; Elbourn, 1966); 4-5 in D. curvirostria (Green, 1956); 5-6 in D. atkinsoni (Green, 1956); 4-6 in D. thomsoni (Green, 1956); 3 in D. ambigua Scourfield (Green, 1956); 4 in D. longispina (Ingle, Wood and Banta, 1937); 4 in D. galeata mendotae Birge (Hall, 1962); 4-5 in D. schødleri (LeSuer, 1959).

#### Size of Females in the First Adult Instar

D. schødleri becomes mature in various instars; thus one might expect that the size of females during the first adult instar (when the first clutch of eggs is laid) may also vary. Anderson (1932) found the mean size of D. magna females during the first adult instar varied from 2.49 to 2.60 mm, the animals being first primiparous in instars six through nine. The size of females during the first adult instar may also vary in different animals under identical conditions, even when these animals all become mature in the same instar (Green, 1954).

Animals maintained at room temperatures fluctuating between 22 and 29.4 C varied in size from 46.0 micrometer units (1.50 mm) to 60.0 micrometer units (1.95 mm) when first mature, irrespective of the instar number. The mean size of females maturing in the fifth instar was 50.5 micrometer units (1.64 mm), and 53.5 micrometer units (1.74 mm) for females maturing in the sixth instar (Table 9). The Student t test was made to determine whether the two mean sizes were significantly different. The calculated t value for the two mean sizes was 6.0061 and is highly significant ( $P < 0.01$ ).

Animals kept in a water bath with temperature maintained at  $20 \pm 1$  C varied in size from 44.3 micrometer units (1.44 mm) to 70.0 micrometer units (2.28 mm) when first mature, regardless of instar number. The mean size of females maturing in the fifth instar was 55.3 micrometer units (1.80 mm), and 55.5 micrometer units (1.81 mm) for females maturing in the sixth instar. The calculated t value for the two mean sizes was 0.1941 and

Figure 16. The relationship between the initial size and the size in the first adult instar of female Daphnia schødleri. The data are based on 57 females that were primiparous in the fifth instar at  $20 \pm 1$  C.

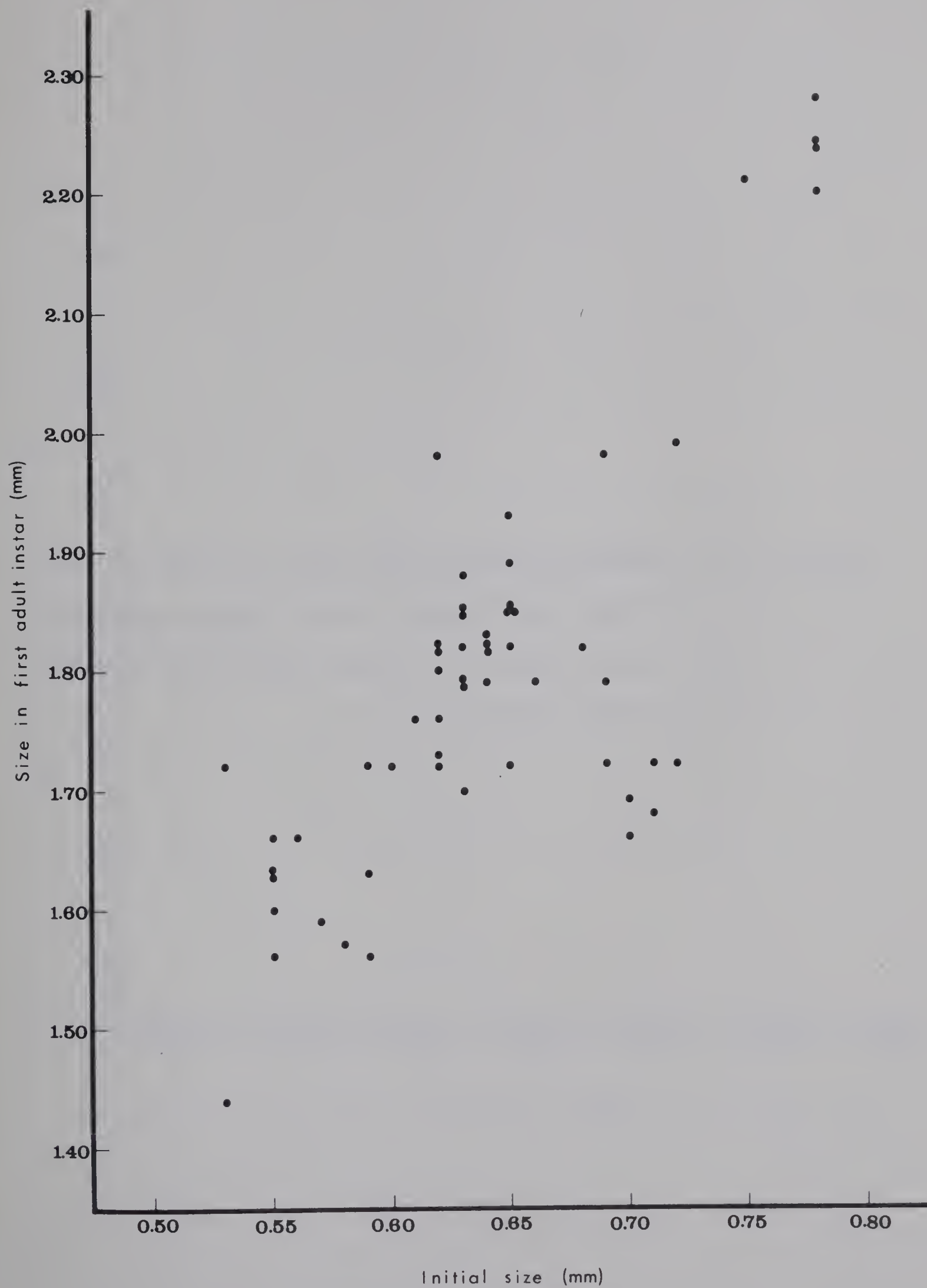
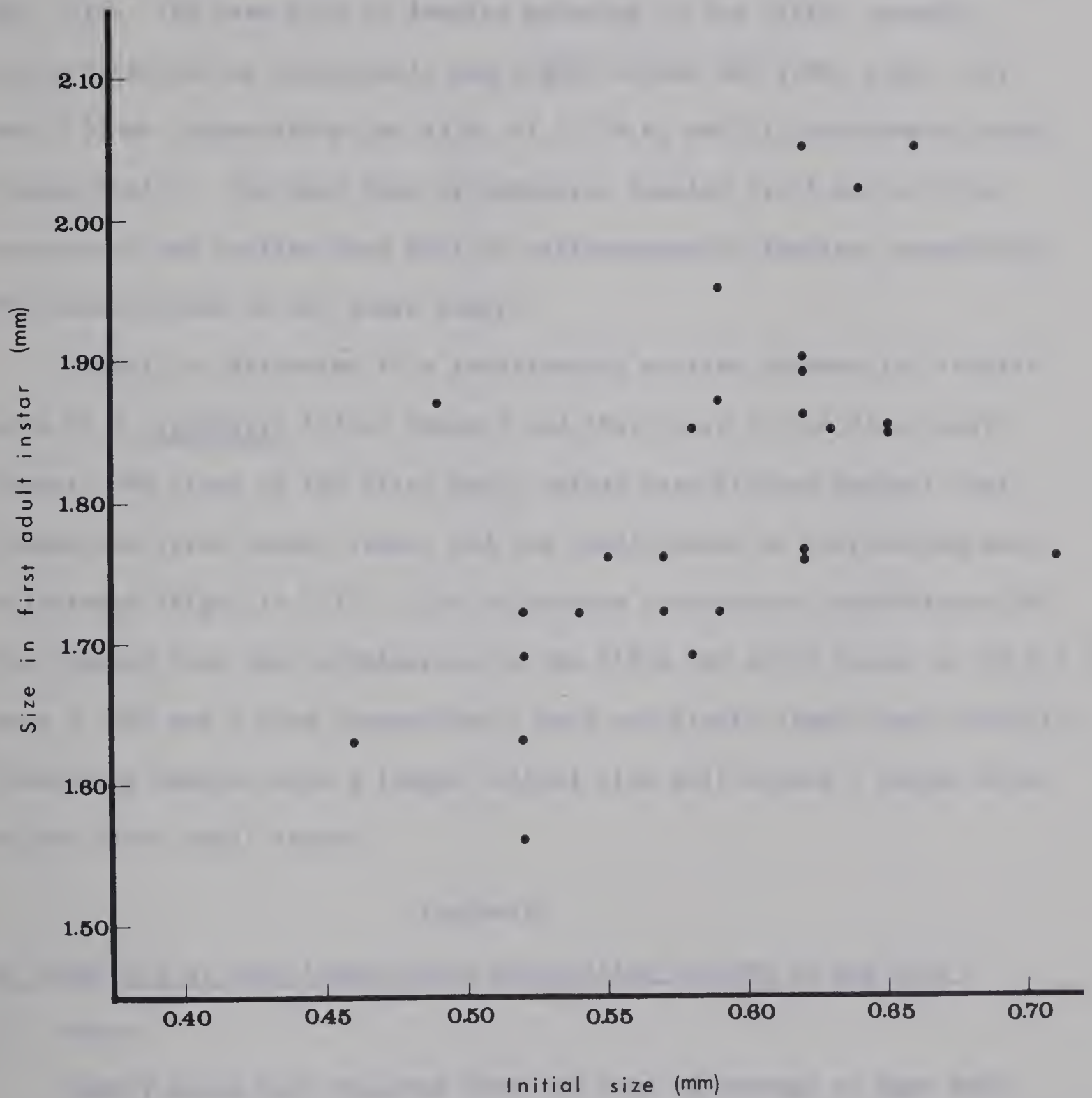


Figure 17. The relationship between the initial size and the size in the first adult instar of female Daphnia schødleri. The data are based on 27 females that were primiparous in the sixth instar at  $20 \pm 1$  C.







is not significant ( $P > 0.05$ ).

For animals kept in a refrigerator with temperature maintained at  $5 \pm 1$  C, the size of females when the first clutch of eggs was laid varied from 57.4 micrometer units (1.87 mm) to 74.0 micrometer units (2.41 mm), irrespective of the instar in which the first clutch of eggs was laid. The mean size of females maturing in the sixth, seventh, seventh (producing ephippium), and eighth instar was 2.08, 2.20, 1.91, and 2.31 mm respectively (or 63.8, 67.7, 58.8, and 71.0 micrometer units respectively). The mean size of ephippial females (1.91 mm) in this experiment was smaller than that of parthenogenetic females, supporting the observations of the field study.

Finally to determine if a relationship existed between the initial size of D. schødleri (first instar) and their size in the first adult instar, the sizes of the first adult instar were plotted against their respective first instar sizes, and the coefficients of correlation were calculated (Figs. 16 & 17). The calculated correlation coefficients for the females that were primiparous in the fifth and sixth instar at  $20 \pm 1$  C were 0.7807 and 0.6364 respectively; both are highly significant ( $P < 0.01$ ), indicating females with a larger initial size will attain a larger size in the first adult instar.

#### Longevity

##### D. schødleri at room temperatures fluctuating between 22 and 29.4 C

###### (1) Males

Twenty males were observed from the time of passage as eggs into the brood pouch until the end of their life. Eight animals lived through 20 instars, and one survived to the 26th instar. Table 10 gives the number of survivors during each instar, and survival curves are illustrated in Figure 18. The maximum longevity for male D. schødleri was 68.07 days,

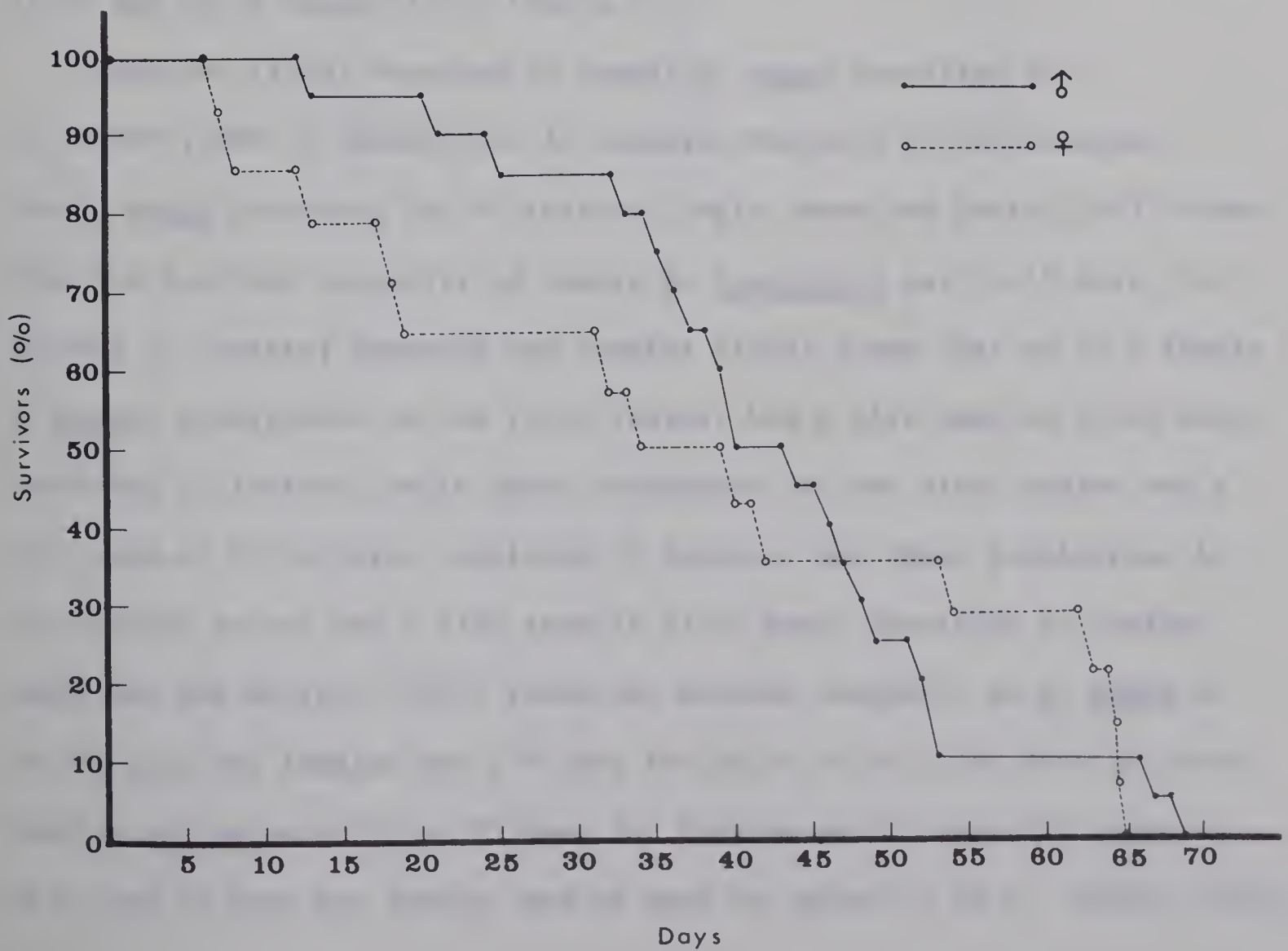
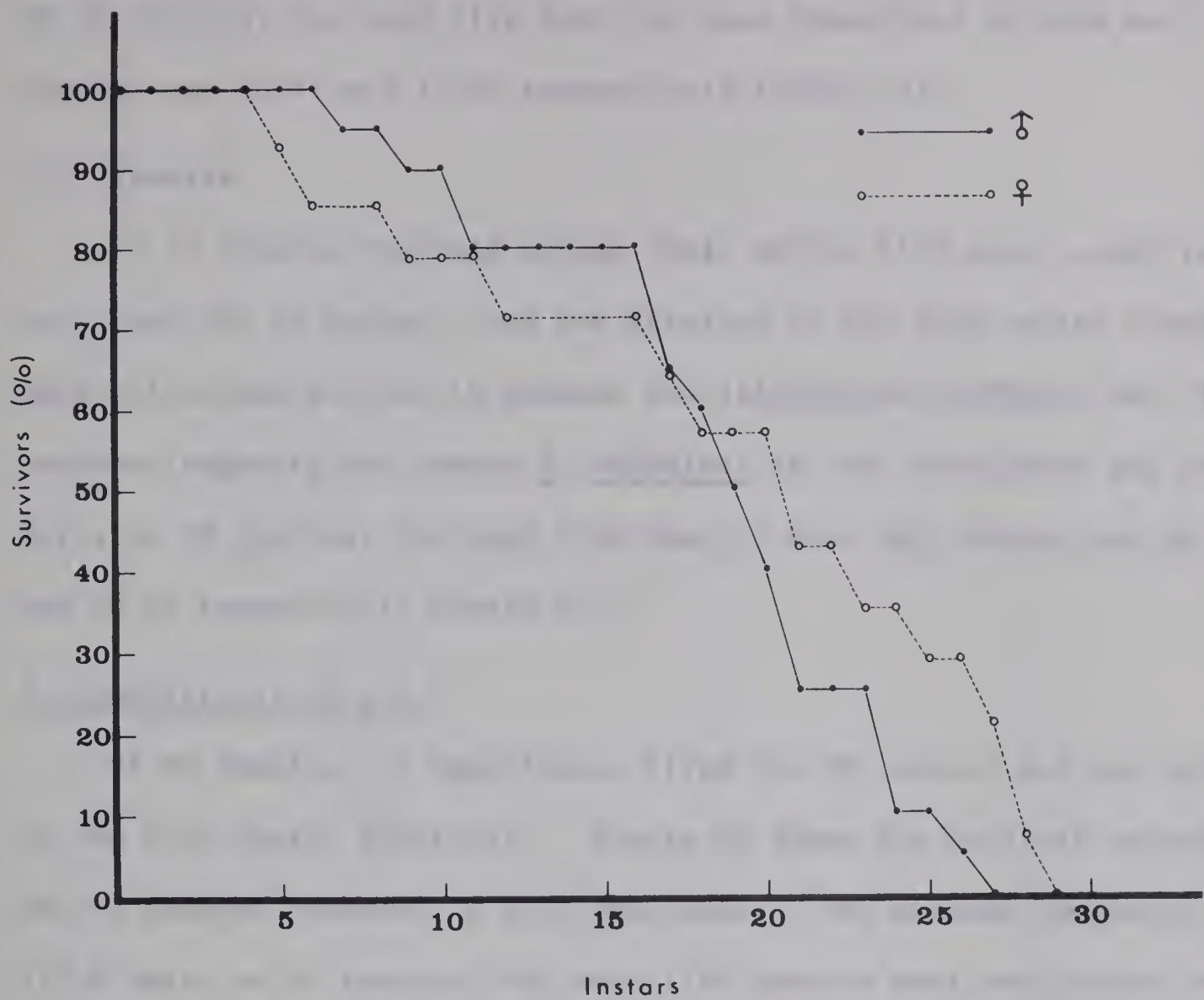


Table 10. The number of surviving male and female Daphnia schødleri during each instar at room temperatures, fluctuating between 22 and 29.4 C.

Instar	Number of survivors	
	Males	Females
1	20	14
2	20	14
3	20	14
4	20	14
5	20	13
6	20	12
7	19	12
8	19	12
9	18	11
10	18	11
11	16	11
12	16	10
13	16	10
14	16	10
15	16	10
16	16	10
17	13	9
18	12	8
19	10	8
20	8	8
21	5	6
22	5	6
23	5	5
24	2	5
25	2	4
26	1	4
27	0	3
28	-	1
29	-	0



Figure 18. Survival curves for male and female Daphnia schødleri.  
Data based on 20 males and 14 females at room temperatures, fluctuating between 22 and 29.4 C. The upper figure is the survival curves in instars; the lower figure is the survival curves of the same animals in days.





or 26 instars; the mean life span (or mean longevity) in days and instars was 40.97 and 17.65 respectively (Table 12).

## (2) Females

Of 14 females observed during their entire life span, eight individual lived for 20 instars, and one survived to the 28th instar (Table 10). Survival curves for the 14 females are illustrated in Figure 18. The maximum longevity for female D. schødleri in this experiment was 64.63 days, or 28 instars; the mean life span in days and instars was 36.42 and 18.21 respectively (Table 12).

### D. schødleri at 20 + 1 C

Of 46 females, 15 individuals lived for 30 instars and two survived to the 41st instar (Table 11). Figure 19 shows the survival curves for the 46 females observed in this experiment. The maximum longevity was 112.6 days, or 41 instars; the mean life span in days and instars was 51.93 and 20.78 respectively (Table 12).

Anderson (1932) observed 30 female D. magna surviving for 14 instars, and 32 animals for 13 instars; Kerherve (1926) observed two D. magna surviving for 24 instars; Ingle, Wood and Banta (1937) found that the greatest longevity of female D. longispina was 51.19 days, involving 23 instars; Anderson and Jenkins (1942) found that at 25 C female D. magna, primiparous in the fifth instar, had a life span of 50.25 days, involving 21 instars, while those primiparous in the sixth instar had a life span of 53.54 days, involving 22 instars, and those primiparous in the seventh instar had a life span of 52.04 days, involving 22 instars. MacArthur and Baillie (1929) found the maximum longevity of D. magna to be 202 days for females and 179 days for males at 8 C; 150 days for both females and males at 10 C; 99 days for females and 92 days for males at 18 C; and 57 days for females and 46 days for males at 28 C. LeSuer (1959)





Table 11. The number of surviving female Daphnia schødleri during each instar at 20  $\pm$  1 C.

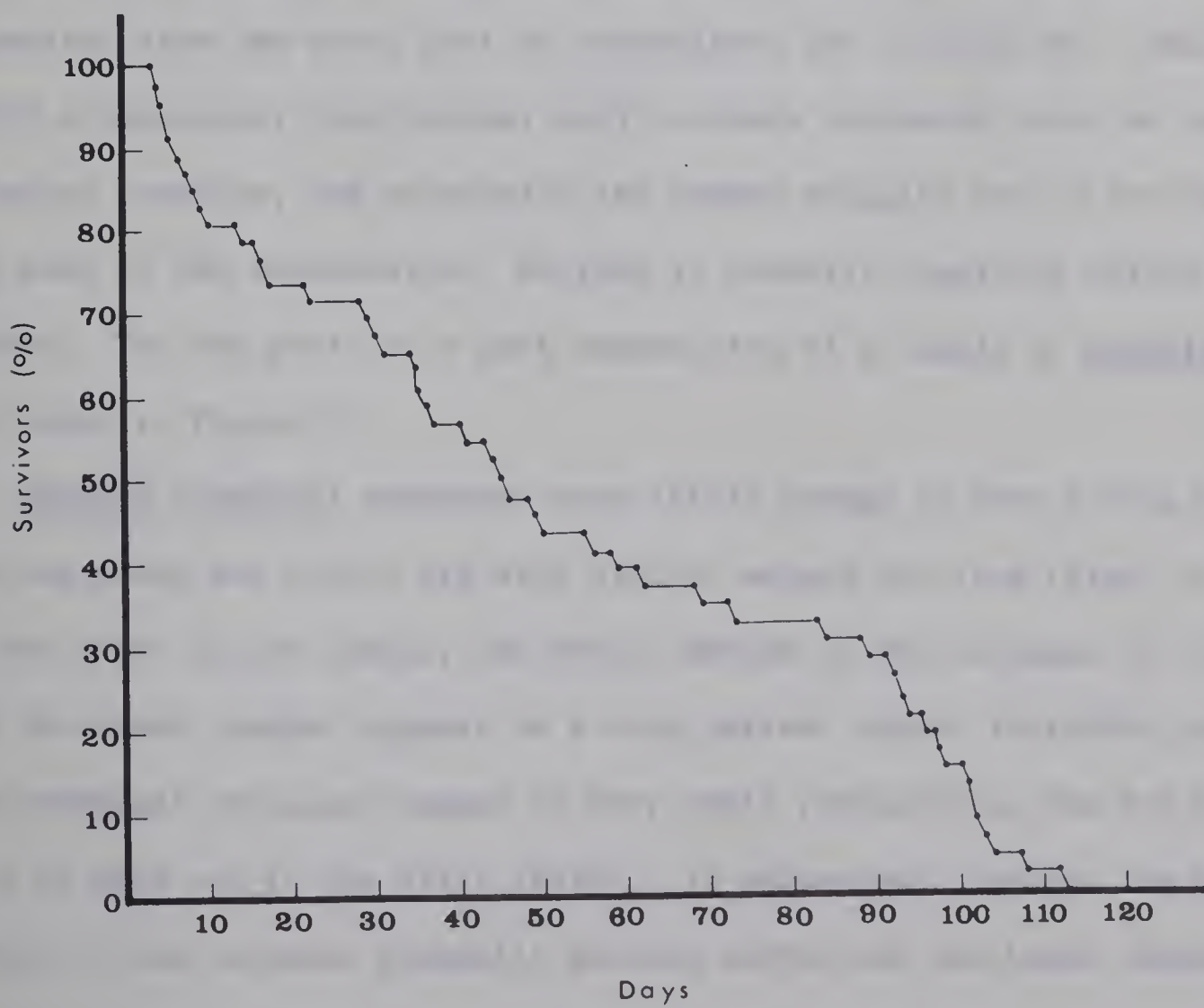
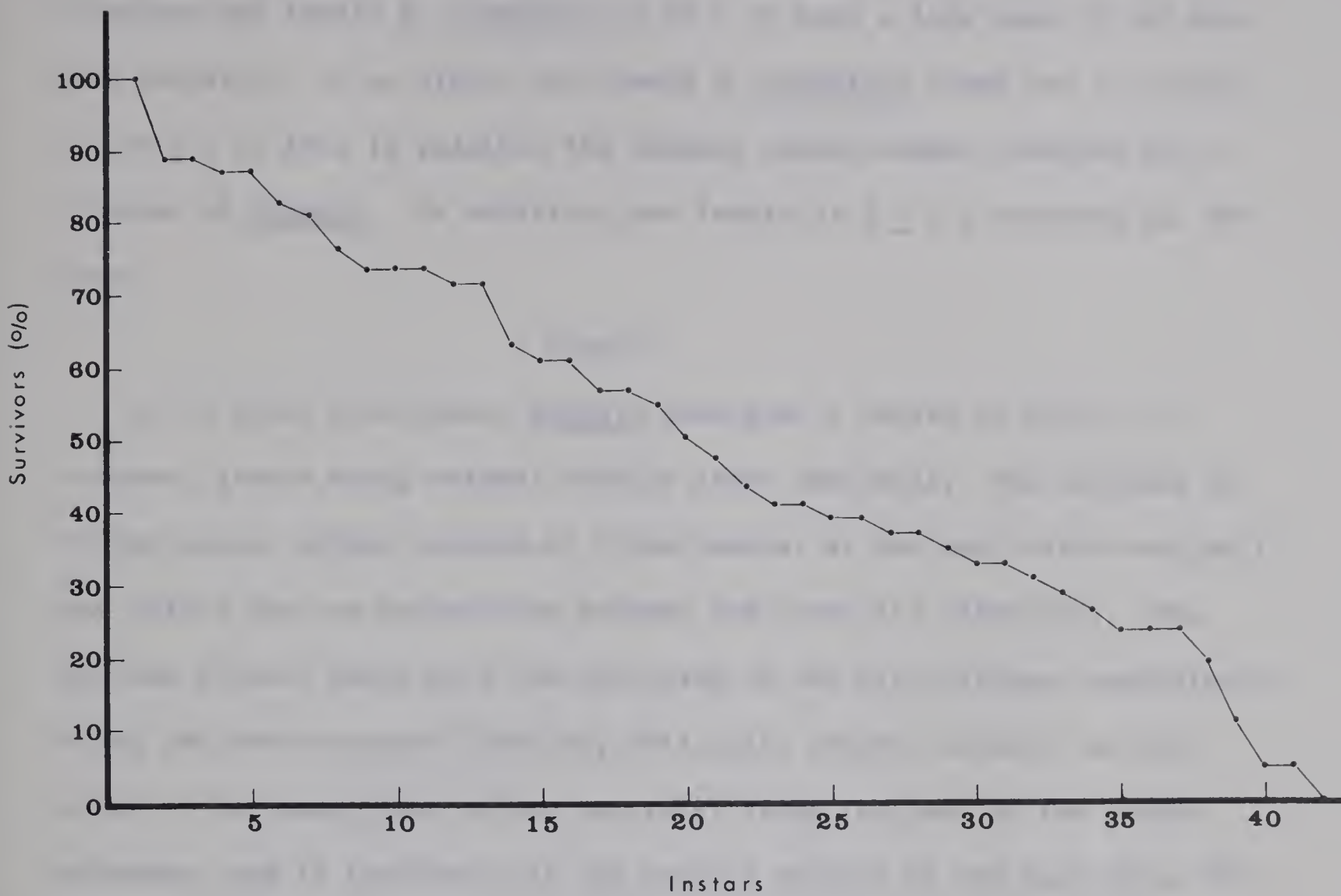
Instar	Number of survivors	Instar	Number of survivors
1	46	22	20
2	41	23	19
3	41	24	19
4	40	25	18
5	40	26	18
6	38	27	17
7	37	28	17
8	35	29	16
9	34	30	15
10	34	31	15
11	34	32	14
12	33	33	13
13	33	34	12
14	29	35	11
15	28	36	11
16	28	37	11
17	26	38	9
18	26	39	5
19	25	40	2
20	23	41	2
21	21	42	0



Table 12. Longevity of Daphnia schødléri.

Temperature	Mean Longevity						Maximum Longevity			
	Males			Females			Males		Females	
	days	instars	days	instars	days	instars	days	instars	days	instar
22-29.4 C	40.9	17.65	36.42	18.21	68.07	26	64.63	28		
20 ± 1 C	-	-	51.93	20.78	-	-	112.60	41		

Figure 19. Survival curves for 46 female Daphnia schødleri at temperature  $20 \pm 1$  C. Upper figure in terms of instars; lower figure in terms of days.







observed one female D. schødleri at 16 C to have a life span of 149 days (26 instars). In my study, two female D. schødleri lived for 41 instars at  $20 \pm 1$  C; this is possibly the highest instar number recorded for a species of Daphnia. In addition, one female at  $5 \pm 1$  C survived for 207 days.

### Growth

As in other arthropods, Daphnia undergoes a series of molts, or ecdyses, growth being evident shortly after each molt. The increase in volume occurs within seconds or a few minutes at the most after each molt and before the new exoskeleton hardens and loses its elasticity. The molting process begin with the splitting of the old chitinous exoskeleton along the head-carapace junction; this split extends forwards on both sides of the head, then curves ventrally along the base of the second antennae, and it terminates at the ventral surface of the head (Fig. 20). Thus, the part of exoskeleton covering the dorsal region of the head separates from the other part of exoskeleton and is cast off. Meanwhile a mid-dorsal longitudinal split extends backwards from the head-carapace junction, and eventually the animal wriggles out of the remaining part of the exoskeleton. Molting is normally completed within a minute. The two parts of a cast exoskeleton of a female D. schødleri are shown in Figure 21.

Daphnia schødleri undergoes very little change in form during growth, and the young and adults are very similar except for size (Figs. 22 & 23). In the first instar female, the dorsal margin of the carapace is straight, and the brood chamber appears as a very narrow, almost invisible strip. The abdominal processes appear as very small projections, and are difficult to make out in the first instar. In subsequent instars, the dorsal margin of the carapace gradually becomes curved and the brood chamber and

Figure 20. A female Daphnia schødleri showing the line of splitting (dotted line) during ecdysis.

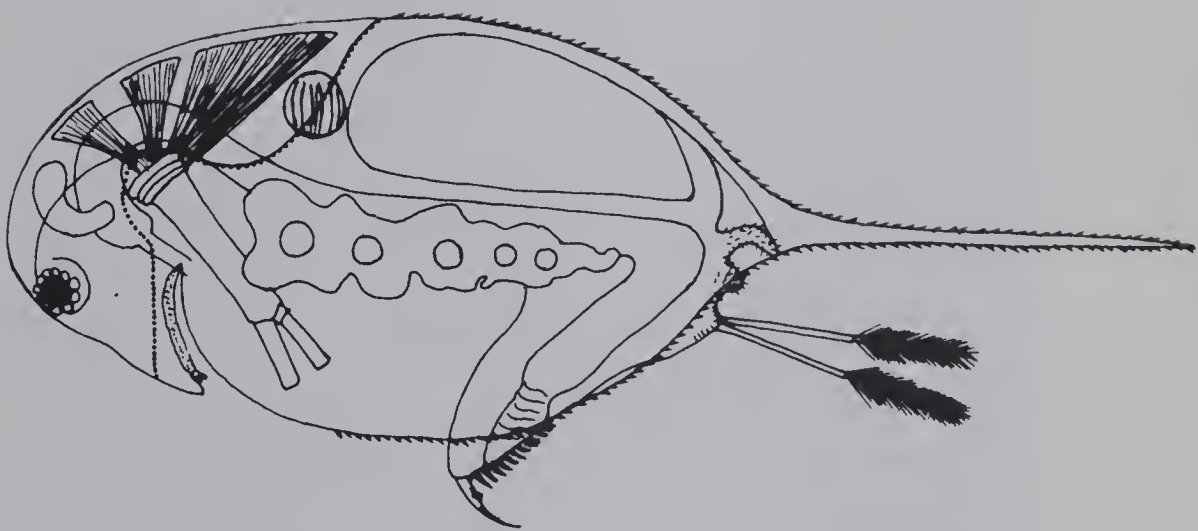


Figure 21. Exoskeleton of a female Daphnia schødleri, showing the two cast parts, i.e. the dorsal head section, and the main body section.



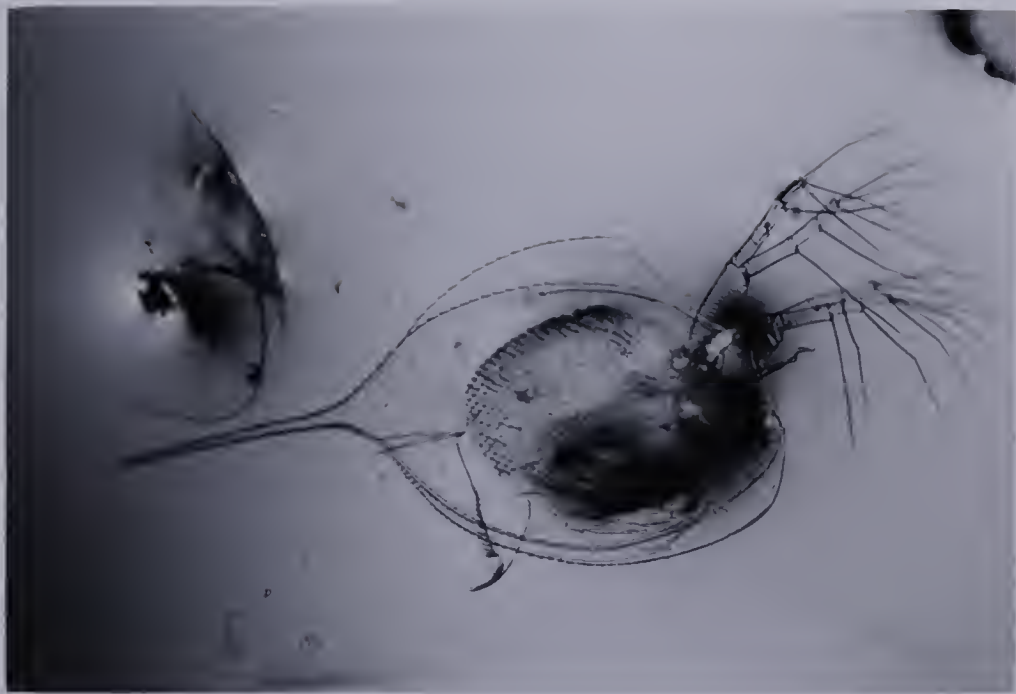


Figure 22. Stages in the growth of a female Daphnia schødleri.  
A. First instar. B. Second instar. C. Third instar.  
D. Fourth instar. E. Fifth instar. F. Sixth instar.  
G. Seventh instar. H. Eighth instar. I. Ninth instar.  
J. Tenth instar. All photographs taken under a compound microscope at 24x magnification.



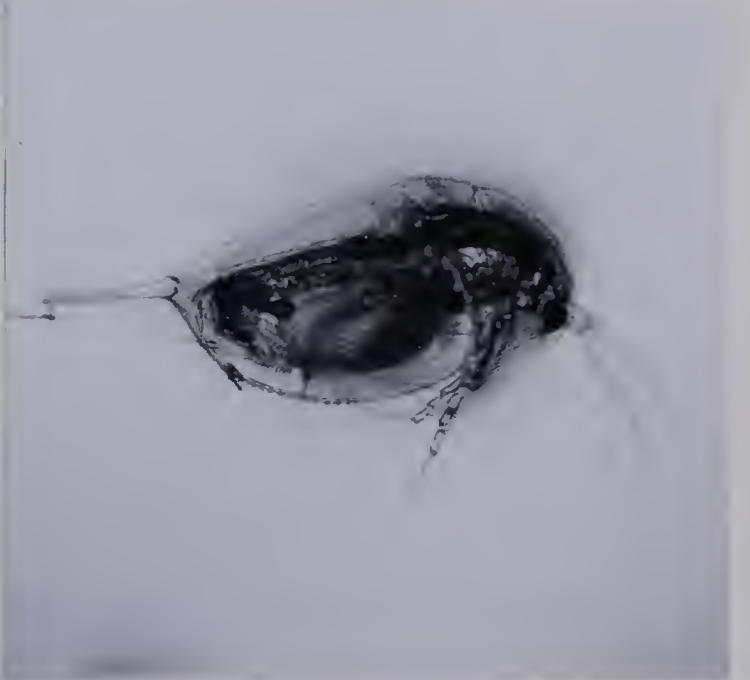
A



B

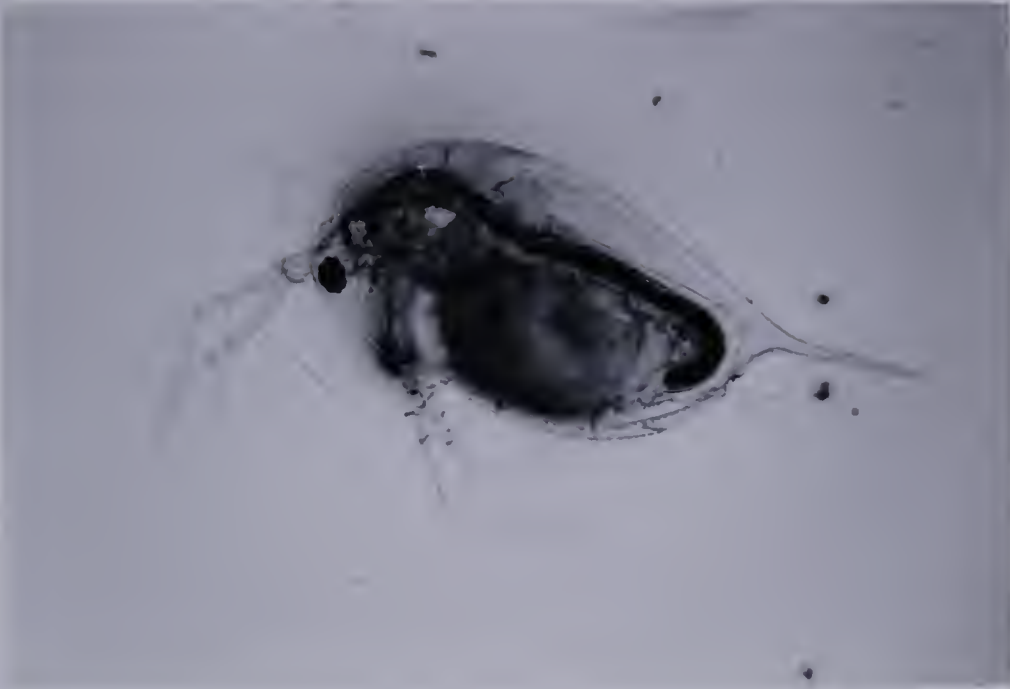


C



D





E



F



G



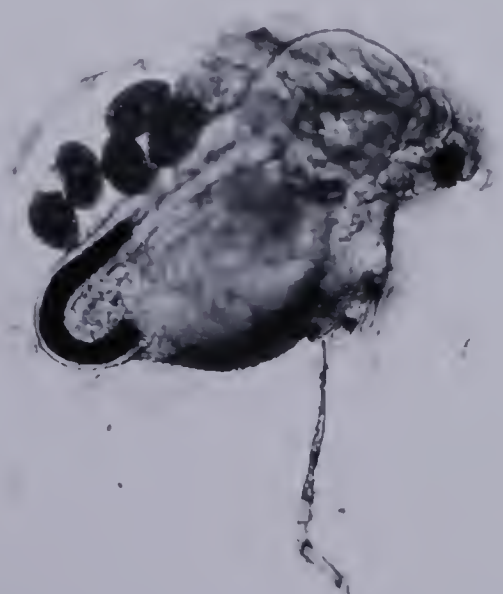




H



I



J

Figure 23. Stages in the growth of one male Daphnia schødleri.  
A. First instar. B. Second instar. C. Third instar.  
D. Fourth instar (the ventral margin of carapace is  
still uniformly curved). E. Fourth instar (the ventral  
margin of carapace is somewhat angular). F. Fifth  
instar. Photographs taken under a compound microscope  
at a magnification of 40x.



A



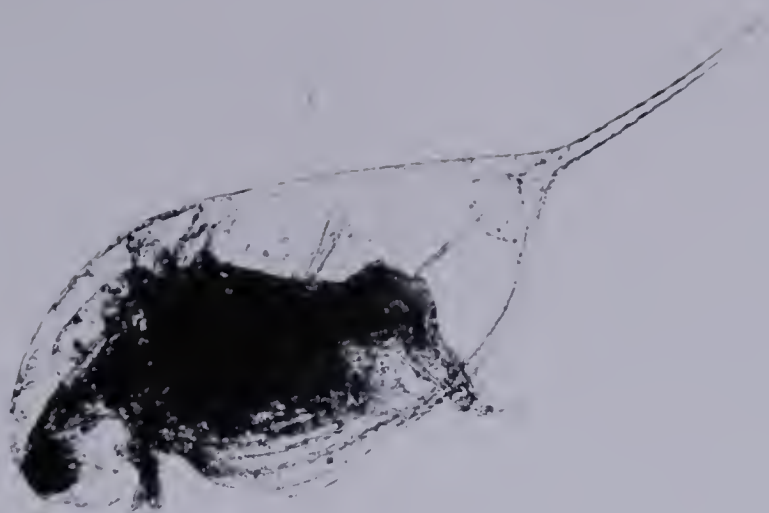
B



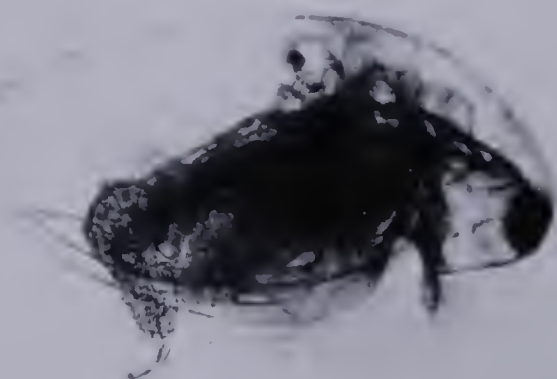
C







D



E



F



abdominal processes increase in size.

During the first and second instars, the male is very similar in appearance to the female, except the male has larger antennules (first antennae). During the first and second instars, the antennules of the male appear as a pair of short stubs with sensory hairs extending from their distal ends; and in subsequent instars the antennules increase in size and length. The only abdominal process apparent in the male is one that corresponds to the second abdominal process of the female. This abdominal process appears as a very small projection during the first and second instars, and it increase in size during subsequent instars. The ventral carapace margin of first and second instar males curves in a fairly uniform fashion, and there are no hair-like projections along the anterior portion (breast margin). In most cases, the hair-like projections (bristles) were first observed in third instar males, but occasionally they were first evident in the fourth instar. In both situations, the ventral margin was still uniformly rounded. The breast margin becomes somewhat angular and with a moderate excavation just posterior to the rostrum region during the fourth or fifth instars (usually the fourth instar). The hook on the first thoracic leg is very small during the first and second instars and becomes distinguishable in the third instar, at which time the first flagellum of the first thoracic leg becomes longer. In subsequent instars both these structures increase in size.

Figure 24 illustrates growth curves for eight females that were primiparous in the sixth instar at room temperatures fluctuating between 22 and 29.4 C. Data used for constructing this figure are given in Appendix 2A. The curves are similar in shape and the points of inflection come immediately before the primiparous instar (sixth instar), i.e. the

Figure 24. Growth curves based on data for eight female Daphnia schødleri that were primiparous in the sixth instar and lived for at least 20 instars at temperatures fluctuating from 22-29.4 C.

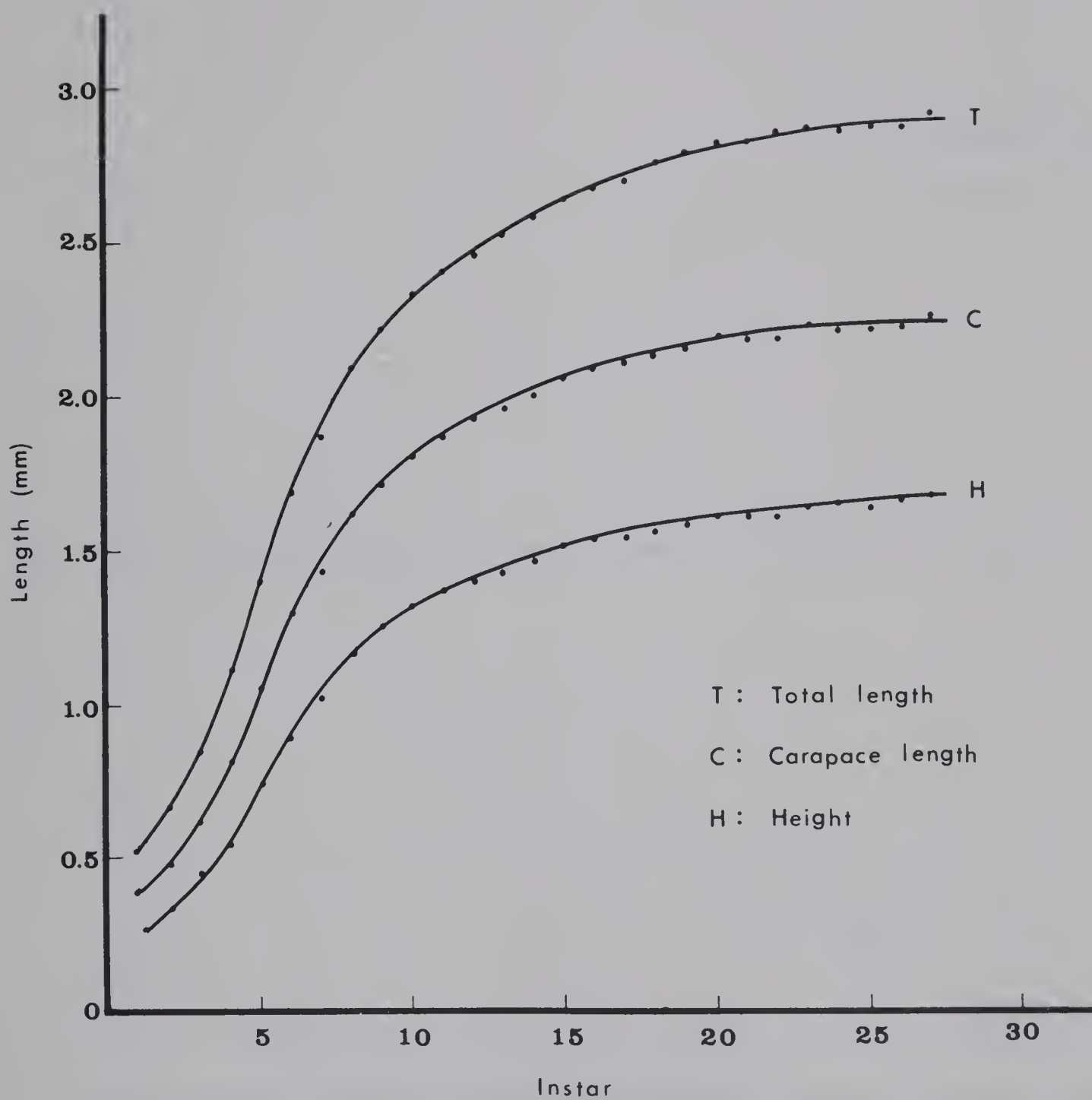
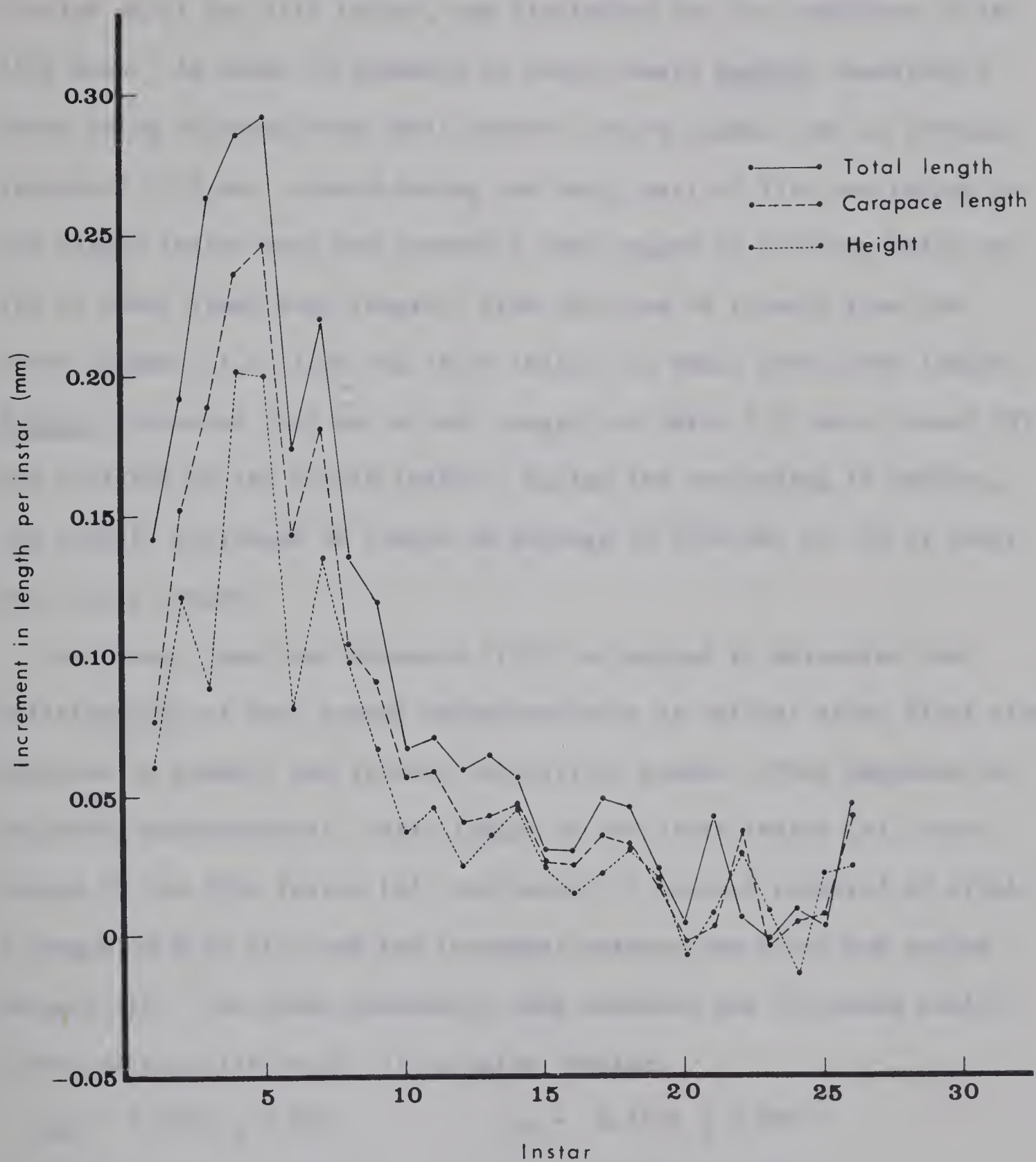


Figure 25. Growth increment curves for the animals of Figure 24.







inflection occurs at a point that corresponds to the time of sexual maturity. Figure 25 shows the growth increments for the same animals as in Figure 24. Data for constructing this figure are given in Appendix 2B. Growth increments increased through the fifth instar, then gradually decreased until the 15th instar, and fluctuated for the remainder of the life span. As shown in Appendix 2A these female Daphnia immediately after being released from their mother's brood chamber had an average length of 0.52 mm. Growth during the early part of life was rapid; by the eighth instar they had reached a body length of 2.09 mm, which was 71% of their final body length. From the time of release from the brood chamber (i.e. from the first instar) to their final body length, Daphnia increased 2.41 mm in body length, of which 1.57 mm or about 65% was attained by the eighth instar. During the succeeding 19 instars, the animals increased in length an average of 0.84 mm, or 35% of their total body length.

Anderson, Lumer and Zupancic (1937) attempted to determine the relationships of such growth characteristics as initial size, final size, duration of growth, and initial velocity of growth. They employed the following measurements: total length in the first instar (a), total length in the 20th instar (A), the number of instars required to attain a length of 0.8A (t), and the increment between the first and second instars (i). For these parameters they obtained the following coefficients of correlation for 47 D. pulex females:

$$\begin{array}{ll} r_{aA} = 0.2309 \pm 0.0931 & r_{at} = 0.1641 \pm 0.0957 \\ r_{ai} = -0.4481 \pm 0.0792 & r_{At} = -0.3893 \pm 0.0835 \\ r_{Ai} = -0.1483 \pm 0.0963 & \end{array}$$

They pointed out that only  $r_{ai}$  and  $r_{At}$  were significantly different from zero. There was thus an indication of an inverse relationship between

Figure 26. Growth curves based on data for eight male Daphnia  
schødleri that lived for 18 or more instars at  
temperatures fluctuating from 22-29.4 C.

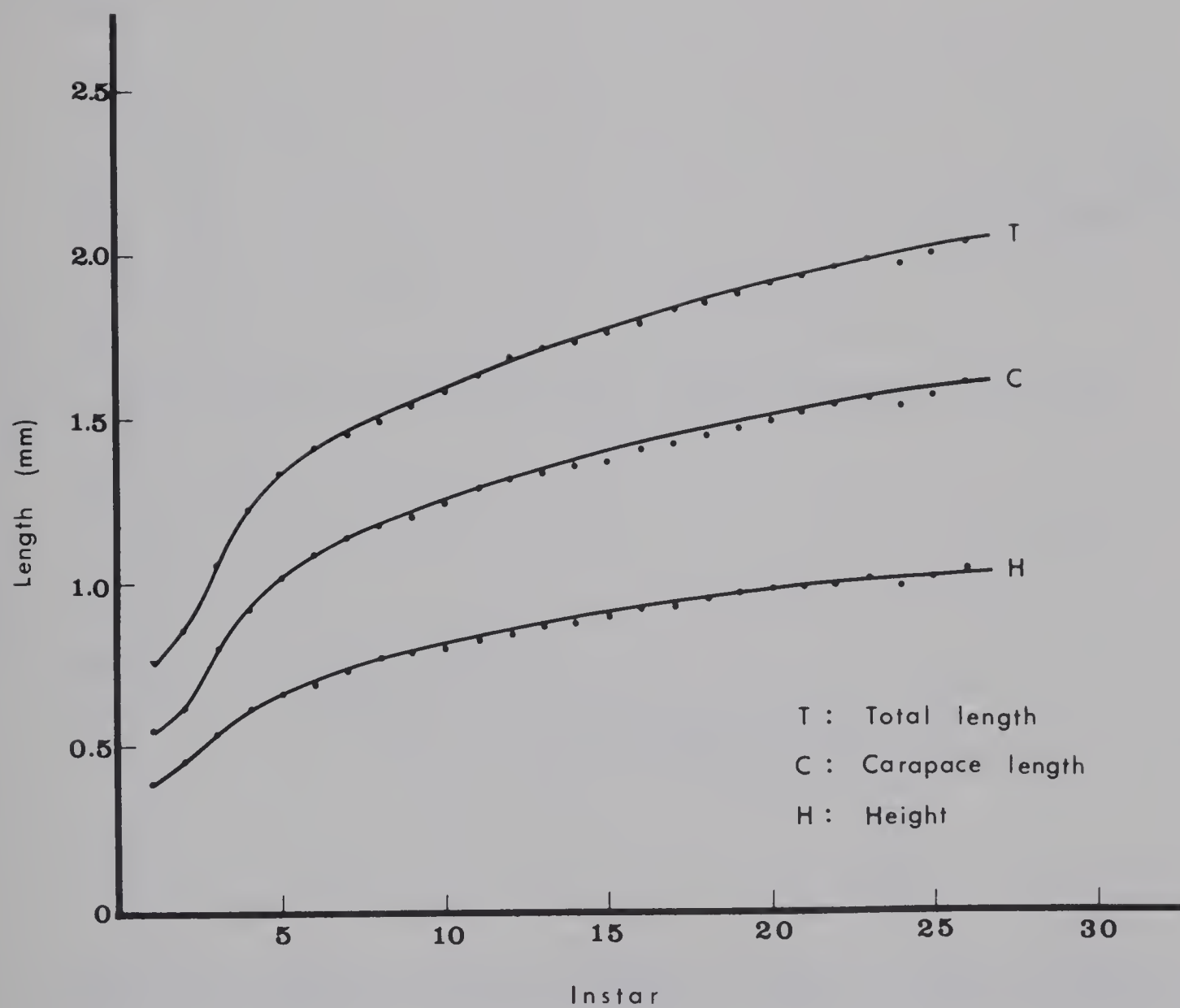
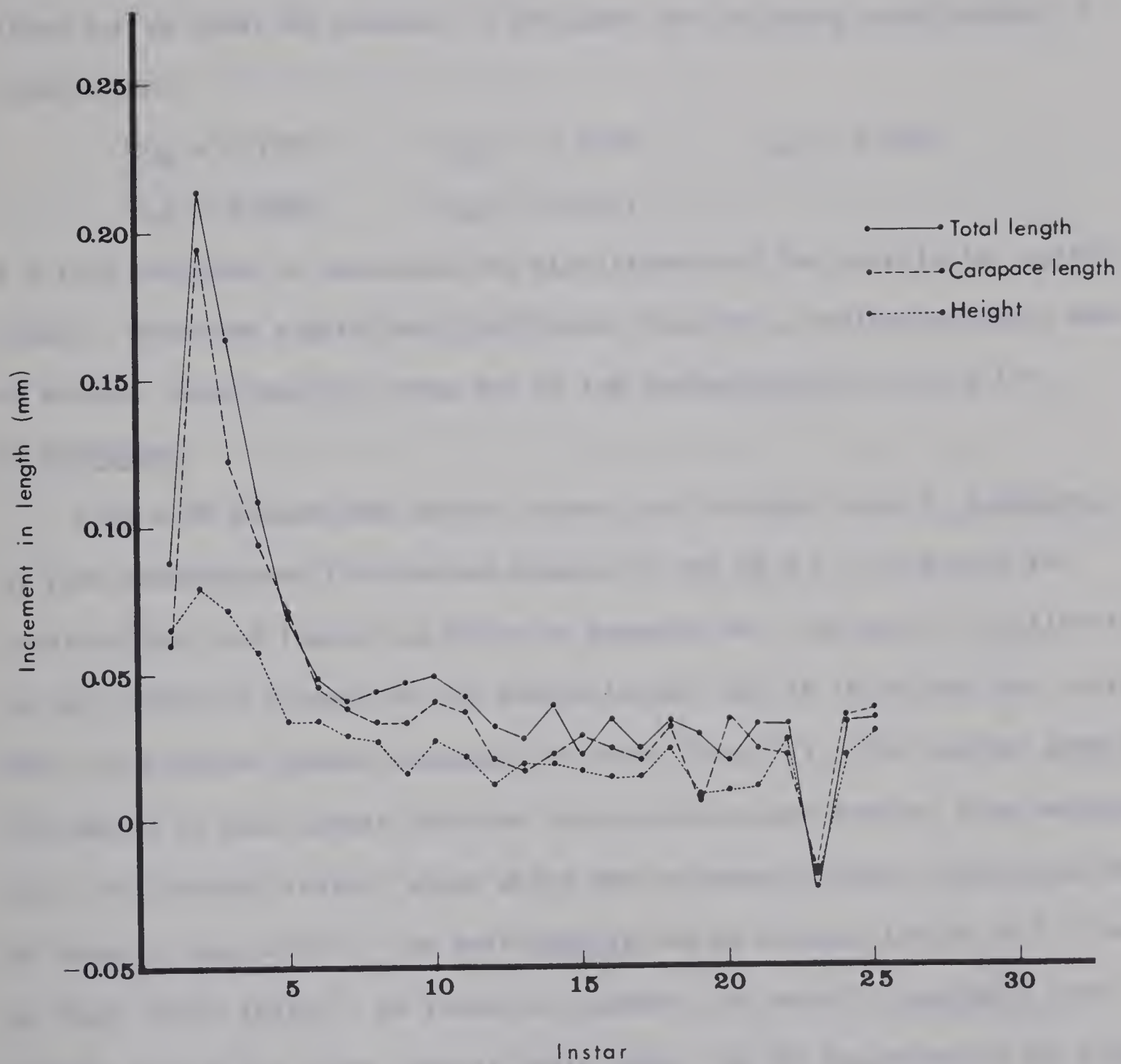


Figure 27. Growth increment curves for male animals of Figure 26.







initial size and initial velocity of growth, and between duration of growth and final size. But there was no apparent relationship between initial and final size, between initial size and duration of growth, or between initial velocity and final size. In my study I performed the same calculations using data from eight female D. schødleri primiparous in the sixth instar and which lived for at least 20 instars. I obtained the following coefficients of correlation:

$$\begin{array}{lll} r_{aA} = 0.1792 & r_{Ai} = 0.3338 & r_{At} = 0.6601 \\ r_{ai} = 0.5965 & r_{at} = -0.5157 & \end{array}$$

A t test was made to determine the significance of the correlation coefficients. None was significantly different from zero, indicating there were no evident relationships among any of the characteristics tested for D. schødleri.

Figure 26 illustrates growth curves for the eight male D. schødleri at room temperatures fluctuating between 22 and 29.4 C. Data used for constructing this figure are given in Appendix 3A. The point of inflection in each curve is located in the second instar, and it is during this instar that the greatest growth increment is found (Fig. 27). The average growth increments of each animal increase through the second instar, then decrease until the seventh instar; after which the increments remain rather constant. As shown in Appendix 3A, the male Daphnia had an average length of 0.76 mm in their first instar. As found for females, the male D. schødleri grew rapidly during the early part of their life. By the beginning of the eighth instar, they had reached a body length of 1.49 mm, which was 73% of their final body length (2.03 mm). In the following 18 instars the male Daphnia grew on the average only 0.54 mm.

The degree of interdependency, if any, between initial size, final size, duration of growth, and initial velocity of growth was also determined for males. Data used for these calculations are based on the six





male animals that lived for 20 or more instars. The following coefficients of correlation were obtained:

$$\begin{aligned} r_{aA} &= 0.5787 & r_{at} &= -0.2501 \\ r_{ai} &= -0.2697 & r_{At} &= 0.5237 \\ r_{Ai} &= -0.0623 \end{aligned}$$

As was found for the female animals, none of the coefficients of correlation was significantly different from zero.

An attempt was made to determine the effect of temperature on the growth of D. schødleri. Brown (1927) and MacArthur and Baillie (1929) found that an increase in temperature, up to sub-lethal levels, increases the initial growth rate of Daphnia by shortening the duration of the instars. This apparently also occurs for D. schødleri (Fig. 29). The growth curve for females at  $20 \pm 1$  C rises steeply for the first 10 days, then ascends gradually for the remainder of the life span; the growth curves plotted at  $5 \pm 1$  C ascend gradually throughout the life span.

MacArthur and Baillie (1929) also concluded that female D. magna at low temperatures increase more slowly in size, but reach a larger final size than females kept at higher temperatures, even though a longer time is required for the females at low temperatures to reach their final size. In my study, observations on growth of females kept at temperatures of  $5 \pm 1$  C were terminated in the 11th or 12th instars; hence their final size is not known. But judging from the growth curves of Figures 28 and 29, it is likely that females at  $5 \pm 1$  C will reach a greater final size than females kept at  $20 \pm 1$  C, with the exception of the ehippial females (Figs. 28 & 29). Females producing ehippia had a slower growth rate than parthenogenetic females, i.e. growth was slower and took place by smaller steps than the growth of parthenogenetic females. This would support the depression hypothesis proposed by Berg (1934), i.e.

Figure 28. Growth curves in instars of female Daphnia schødleri at  $20 \pm 1$  C and  $5 \pm 1$  C.

- A. Data from six females primiparous in the sixth instar at  $5 \pm 1$  C.
- B. Data from 10 females primiparous in the seventh instar at  $5 \pm 1$  C.
- C. Data from two females primiparous in the eighth instar at  $5 \pm 1$  C.
- D. Data from 31 females primiparous in the fifth instar at  $20 \pm 1$  C.
- E. Data from three females primiparous in the seventh instar at  $5 \pm 1$  C, but which produced ephippia instead of parthenogenetic eggs.

Data used to construct these curves are given in Appendices 4 and 5.



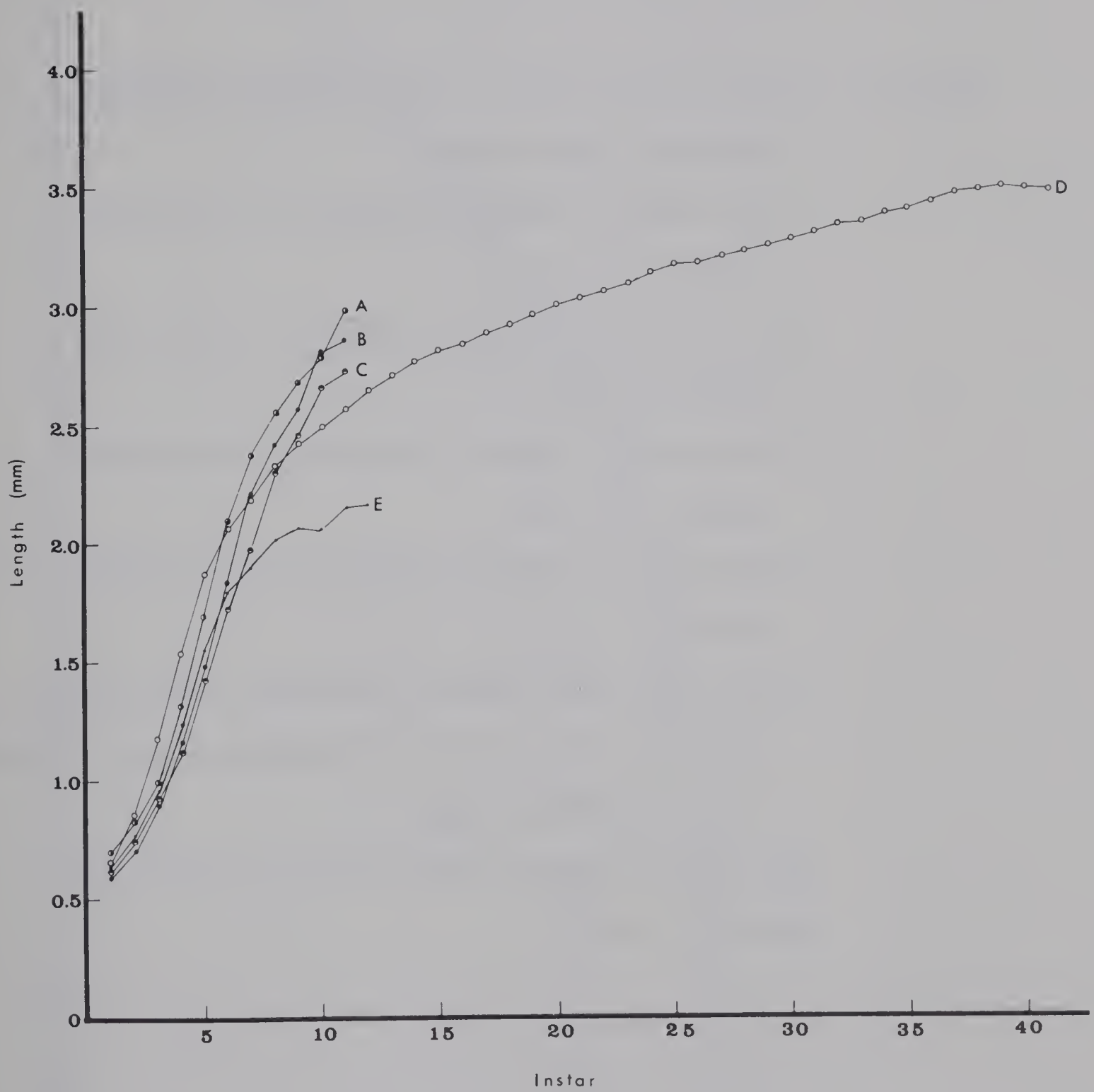


Figure 29. Growth curves in days of female Daphnia schødleri at different temperatures.

- A. Data from 31 females primiparous in the fifth instar at  $20 \pm 1$  C.
- B. Data from six females primiparous in the sixth instar at  $5 \pm 1$  C.
- C. Data from 10 females primiparous in the seventh instar at  $5 \pm 1$  C.
- D. Data from two females primiparous in the eighth instar at  $5 \pm 1$  C.
- E. Data from three females primiparous in the seventh instar at  $5 \pm 1$  C, but which produced ephippia instead of parthenogenetic eggs.

Data used to construct these curves are given in Appendices 6 and 7.

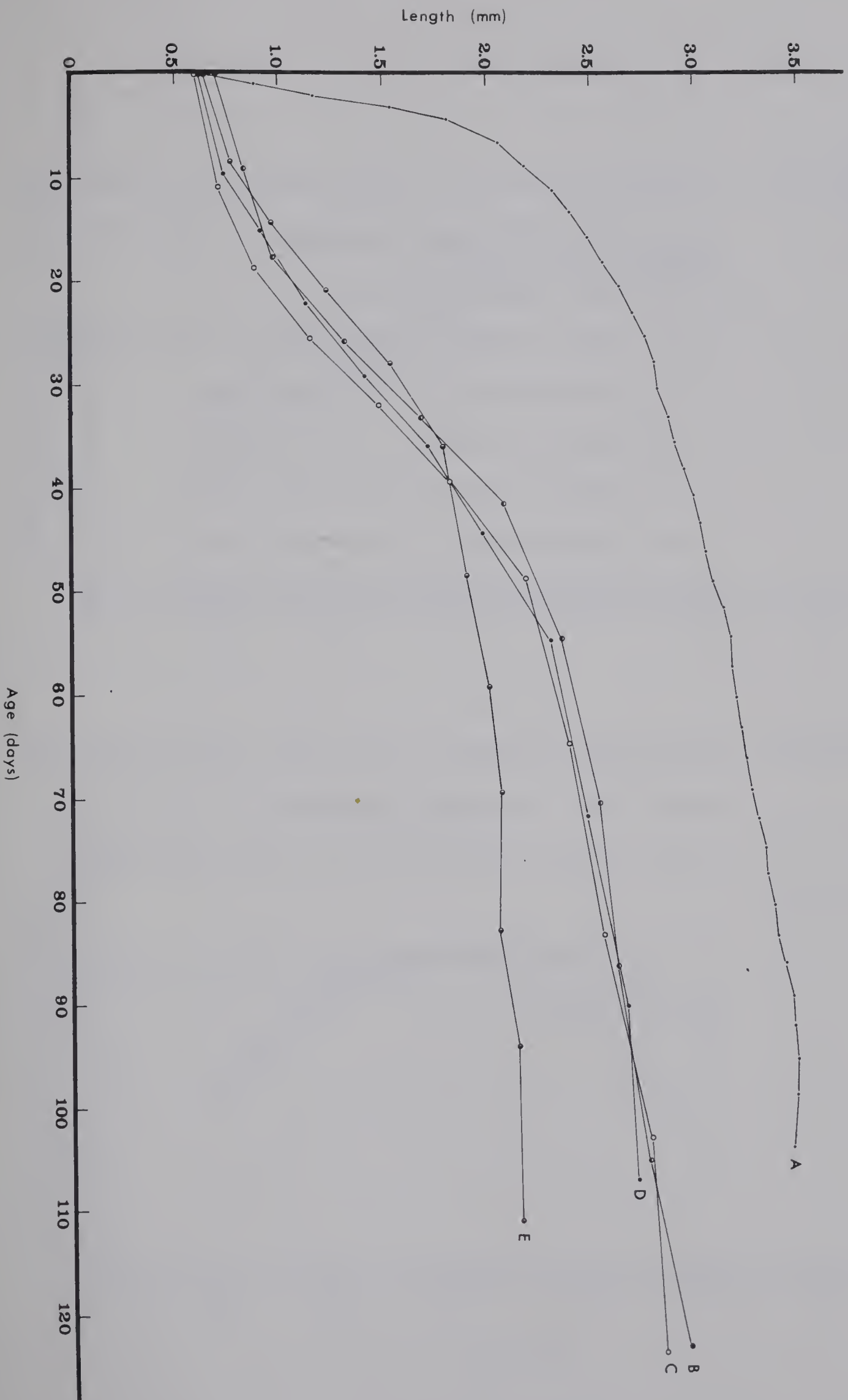


Figure 30a. Mean growth increments of pre-adult instars and first adult instar for Daphnia schødleri at temperatures fluctuating from 22-29.4 C.

- A. Two females mature (primiparous) in the fifth instar.
- B. Ten females mature in the sixth instar.
- C. One female mature in the sixth instar.
- D. Six females mature in the fifth instar.
- E. Two females mature in the sixth instar.
- F. Nine females mature in the sixth instar.

Figure 30b. Mean growth increments of pre-adult instars and first adult instar for Daphnia schødleri at  $20 \pm 1$  C. All lines represent females mature in the fifth instar.

Data for constructing curves are given in Appendices 8A, 8B, and 8C.

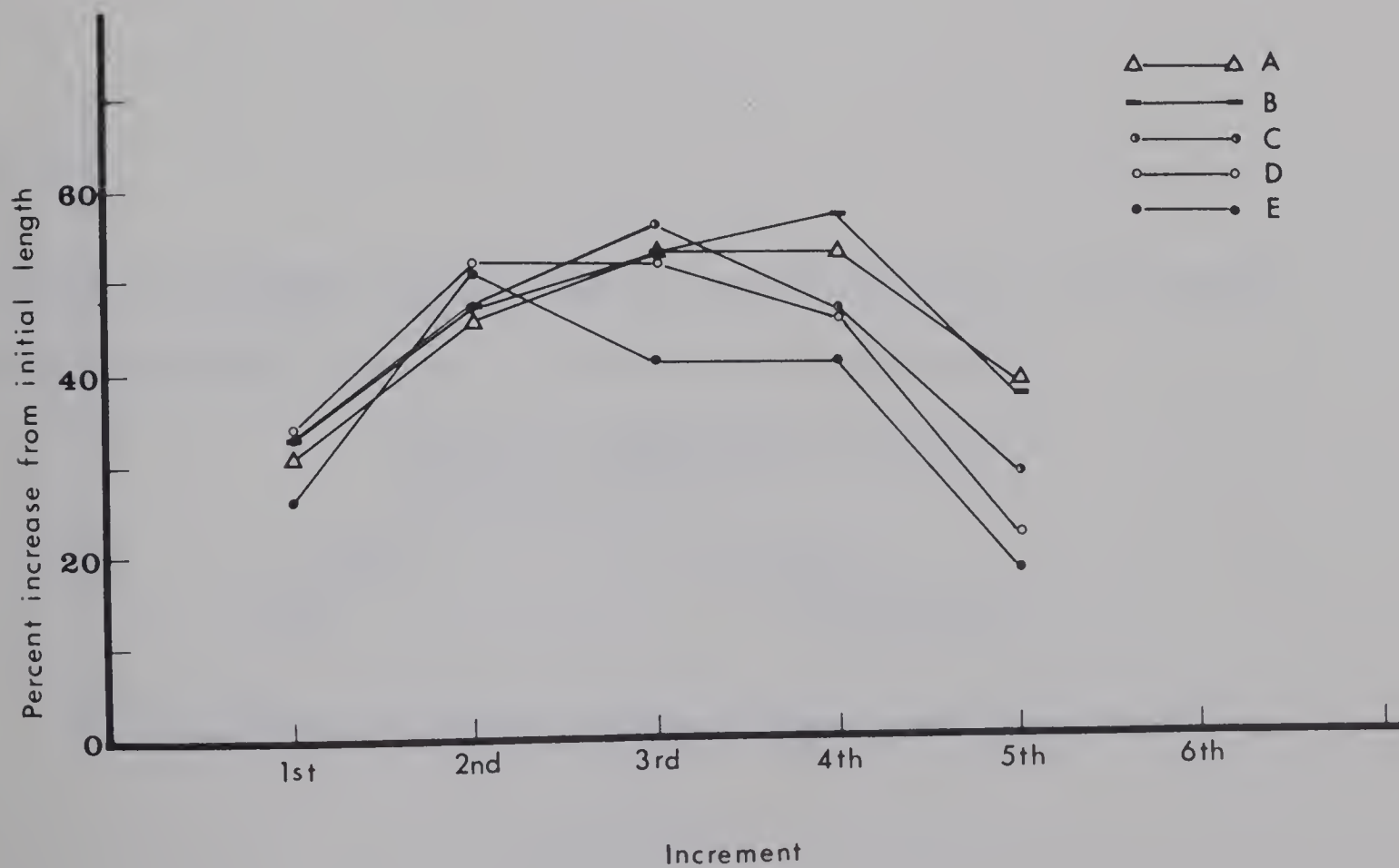
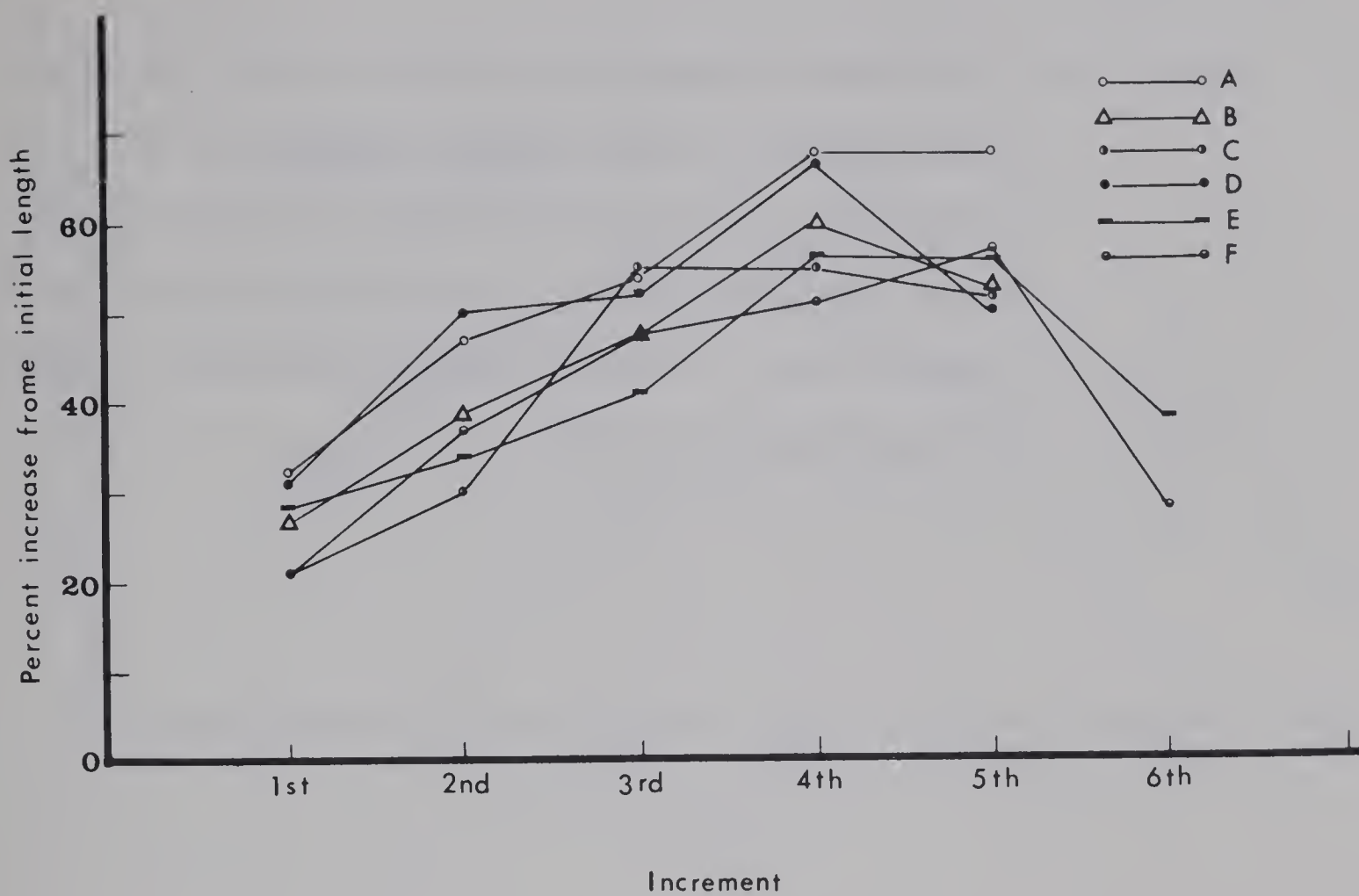
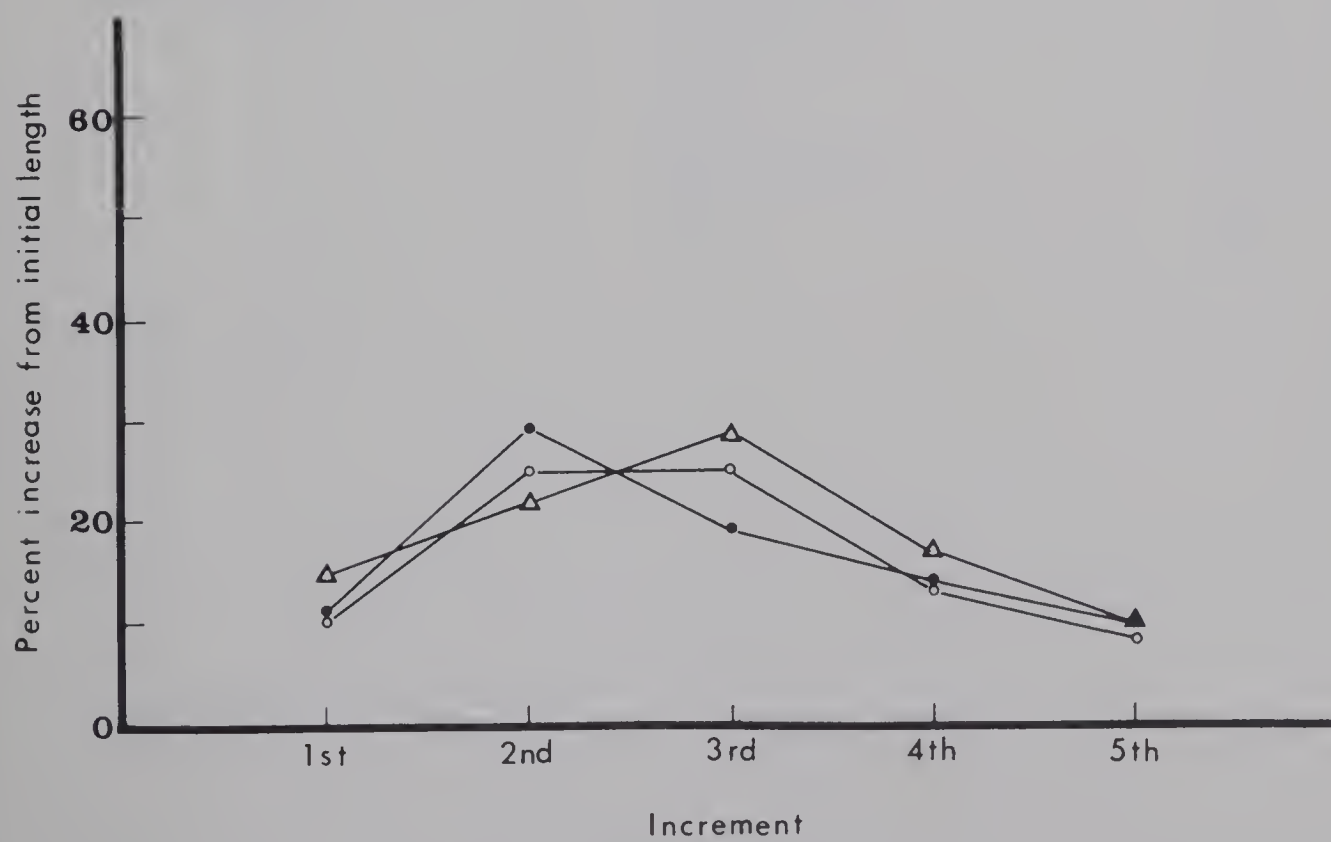
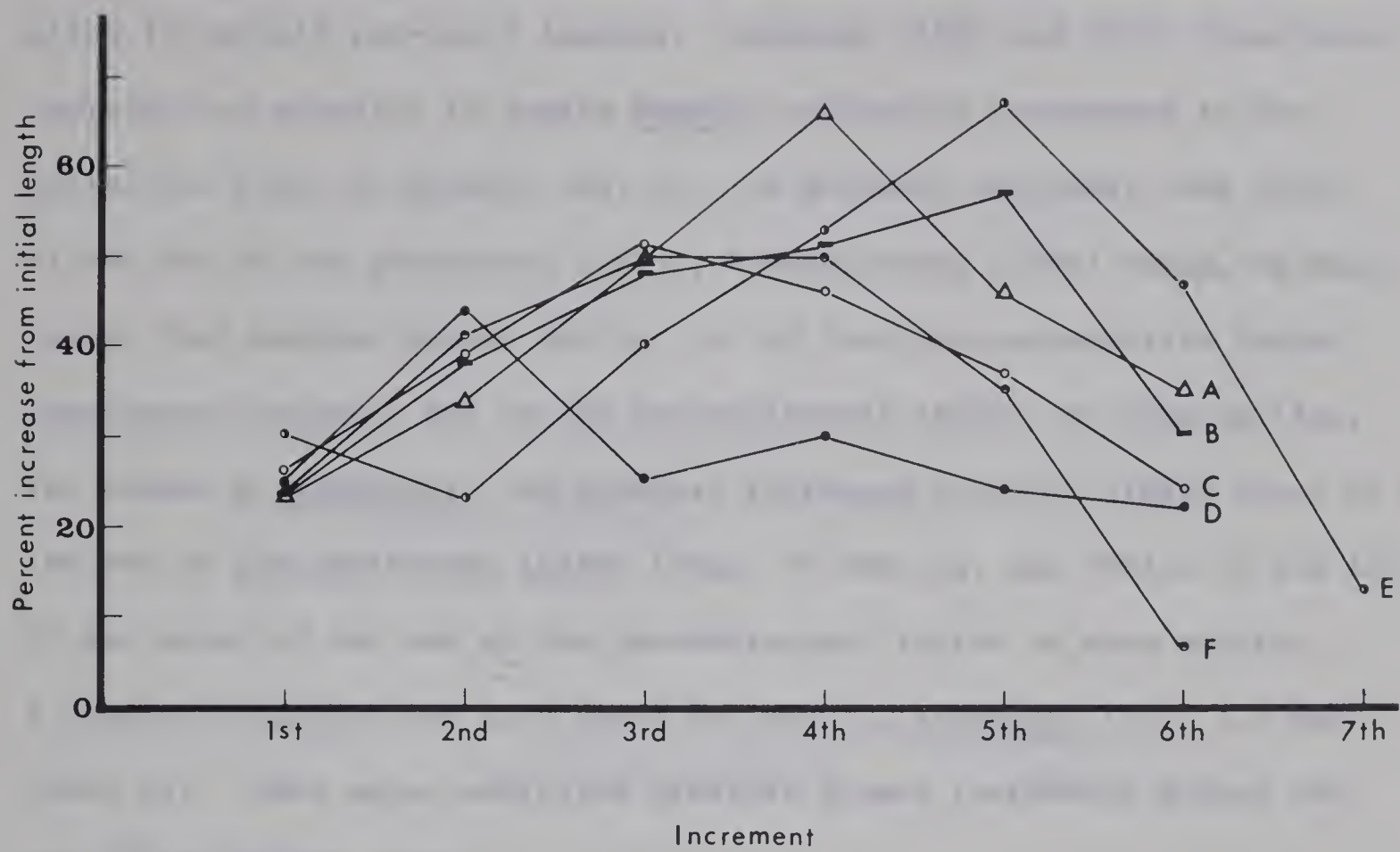


Figure 31a. Mean growth increments of pre-adult instars and first adult instar for female Daphnia schødleri at  $20 \pm 1$  C. A, B, C, D, E represent individuals all mature in the sixth instar; F represents one individual mature in the seventh instar. Data for constructing curves are given in Appendices 8B and 8C.

Figure 31b. Growth increments for male Daphnia schødleri at temperatures fluctuating from 22-29.4 C. Data for constructing curves are given in Appendices 9A and 9B.







the ehippial females are in a state of depression.

It appears that the most intense period of growth in Daphnia takes place in certain pre-adult instars. Anderson (1932 and 1937) found that reproductive maturity in female Daphnia ordinarily correspond to the inflection point in growth, that is, the greatest increment took place at the end of the adolescent instar; however Green (1956) found, in many cases, that maximum growth was not in the last pre-reproductive instar (adolescent instar), but in the pre-adolescent instar, or even earlier. For female D. schødleri, the greatest increment does not always occur at the end of the adolescent instar (Figs. 30 and 31a, and Tables 13 and 14). It may occur at the end of the pre-adolescent instar or even earlier. A similar situation was also found for male D. schødleri (Fig. 31b and Table 15). Most males exhibited greatest growth increments during the end of the second instar.



Table 13. Instar in which greatest growth increment occurred for female Daphnia schødleri at temperatures fluctuating between 22 and 29.4 C.

Primiparous instar	Instar of greatest growth increment	Number of individuals
5th instar	4th	5
	4th & 5th	2
6th instar	3rd & 4th	1
	4th	10
	4th & 5th	2
	5th	9





Table 14. Instar in which greatest growth increment occurred for female Daphnia schødleri at 20  $\pm$  1 C.

Primiparous instar	Instar of greatest growth increment	Number of individuals
5th instar	2nd	7
	2nd & 3rd	4
	3rd	33
	3rd & 4th	4
	4th	7
6th instar	2nd	1
	3rd	7
	3rd & 4th	3
	4th	6
	5th	10
7th instar	5th	1



Table 15. Instar in which greatest growth increment occurred for male Daphnia schødleri at temperatures fluctuating between 22 and 29.4 C.

Instar of greatest growth increment	Number of individuals
2nd	14
2nd & 3rd	1
3rd	1



### Duration of Instars

The average duration of different instars for female D. schødleri at  $20 \pm 1$  C and  $5 \pm 1$  C is given in Table 16 and 17 respectively. For animals reared at  $20 \pm 1$  C, the average time spent in each instar increased with age. This agrees with the results of Ingle, Wood and Banta (1937) for D. longispina; Anderson and Jenkins (1942) for D. magna; and LeSuer (1959) for D. schødleri. Instar one through four (the pre-adult instars) were passed rather rapidly, with instar four (the adolescent instar) being generally longer than the other pre-adult instar. Instar durations for adult animals were much longer than those of pre-adult animals, adult instars varying from 53 to 126 hours.

MacArthur and Baillie (1929) and Hall (1964) found that low temperatures tended to increase the duration of instars in D. magna and D. galeata mendotae respectively. This is also true for D. schødleri of Big Island Lake, with the duration of the first instar of all animals studied being longer than any of the following juvenile instars at  $5 \pm 1$  C. This was due to the sudden temperature change in culture conditions, viz. 20 C to  $5 \pm 1$  C; the young Daphnia used for low temperature study being hatched from parthenogenetic eggs at 20 C and then transferred to 5 C. For purposes of comparison, however, two individuals hatched from parthenogenetic eggs at  $5 \pm 1$  C were also studied through the seventh instar (Table 18). These two animals also show a pattern of growth similar to animals reared under  $20 \pm 1$  C, i.e. instar durations tend to increase with age. The adolescent instar (sixth instar in this case) is longer than any pre-adult instars.





Table 16. Duration of instars in hours for female Daphnia schødleri primiparous in the fifth instar at  $20 \pm 1$  C.

Instar	Mean duration of instar hours	Accumulated time hours	Number of observation
B*	53.19		31
1	22.83	76.02	22
2	24.92	100.94	26
3	24.48	125.42	26
4	33.96	159.38	26
5	52.98	212.36	31
6	53.45	265.81	24
7	53.27	319.08	24
8	55.98	375.06	24
9	56.43	431.49	24
10	55.60	487.09	24
11	57.75	544.84	24
12	59.27	604.11	24
13	59.67	663.78	24
14	60.41	724.19	22
15	63.33	787.52	22
16	60.91	848.43	20
17	62.15	910.58	20
18	61.94	972.52	20
19	62.93	1035.45	20
20	64.65	1100.10	20
21	66.35	1166.45	20
22	66.30	1232.75	19
23	65.71	1298.46	19
24	66.63	1365.09	19
25	68.08	1433.17	18
26	70.85	1504.02	17
27	70.87	1574.89	17
28	69.62	1644.51	16
29	73.20	1717.71	16
30	67.93	1785.64	15
31	69.62	1855.26	15
32	68.06	1923.32	14
33	68.63	1991.95	12
34	68.24	2060.19	11
35	69.41	2129.60	11
36	72.84	2202.44	11
37	71.29	2273.73	9
38	79.55	2353.28	7
39	77.74	2431.02	5
40	125.74	2556.76	2

\* denotes brooding period.



Table 17. Duration of instars in days for Daphnia primiparous in different instars at 5 ± 1 C.  
First adult instars in italics.

Instar	Sixth instar			Seventh instar			Eighth instar		
	Mean duration of instar days	Number of individuals	Mean duration of instar days	Mean duration of instar days	Number of individuals	Mean duration of instar days	Mean duration of instar days	Number of individuals	Number of individuals
1	9	6	8.3	10.7	3	10	9.5	2	2
2	8.7	6	6	8.1	3	10	5.5	2	2
3	8.2	6	6.3	6.7	3	10	7	2	2
4	7.2	6	7.3	6.5	3	10	7	2	2
5	8.5	6	8	7.6	3	10	7	2	2
6	<u>13.2</u>	6	12.7	9.4	3	10	8.5	2	2
7	16	4	<u>10.7*</u>	<u>16</u>	3	10	10.5	2	2
8	15.7	3	10.3	18.7	3	6	<u>17</u>	2	2
9	19	2	13.5*	19.4	2	5	18.5	2	2
10	18	2	11	21	1	1	17	2	2
11	-	-	17*	-	1	-	-	-	-

\* indicates ephippia were produced in this instar.



Table 18. Mean instar duration of two female Daphnia schødleri hatched from parthenogenetic eggs produced at  $5 \pm 1$  C.

Instar	Mean duration of instar days
1	5
2	7
3	8
4	8
5	11.5
6*	12
7	17.5

\* indicates the adolescent instar.





### Size Frequency Distribution

Most studies of growth in Cladocera have been made by analysis of size-frequency distributions in natural populations. A commonly used method consists of plotting the number of individuals against size. In the resulting graph a number of size modes appear that are taken as representative of growth stages or instars. I compared the results secured by this method with results secured by observations on individually reared animals (Fig. 32). Values for the total length frequency during the first instar for all 32 female D. schødleri observed were plotted, and then values for the second instar and so on, up to and including the seventh instar. A composite curve for all the values, which are equivalent to the length frequency of Daphnia present in a sample of natural population, was then plotted. The separate instar curves are shown as broken lines and the composite curve as a solid line. The vertical lines in the upper part of the figure represent the mean total length for each instar up to and including the seventh.

It is obvious that each mode in the composite curve does not represent the mean total length for an instar. For the first four instars, each instar can be easily recognized from a correspondingly distinct mode in the composite curve; whereas the later instars are not so clear cut. Considerable overlapping occurs for the values of various instars.

A similar figure was constructed for 24 males (Fig. 33). Here also it is apparent that each mode in the composite curve does not represent the mean total length for each instar, except for the first instar. For the first four instars, each instar has a corresponding size mode in the composite curve; whereas the last three instars are undistinguishable in the composite curve. Considerable overlapping occurs for the values of

Figure 32. Size frequency distributions during the first seven instars of 32 female Daphnia schødleri reared at temperatures fluctuating from 22-29.4 C. Broken lines designate the actual individual instars. The solid line is a composite curve for all instars. The vertical bars near the upper edge of the figure represent the mean total lengths for each of the first seven instars.

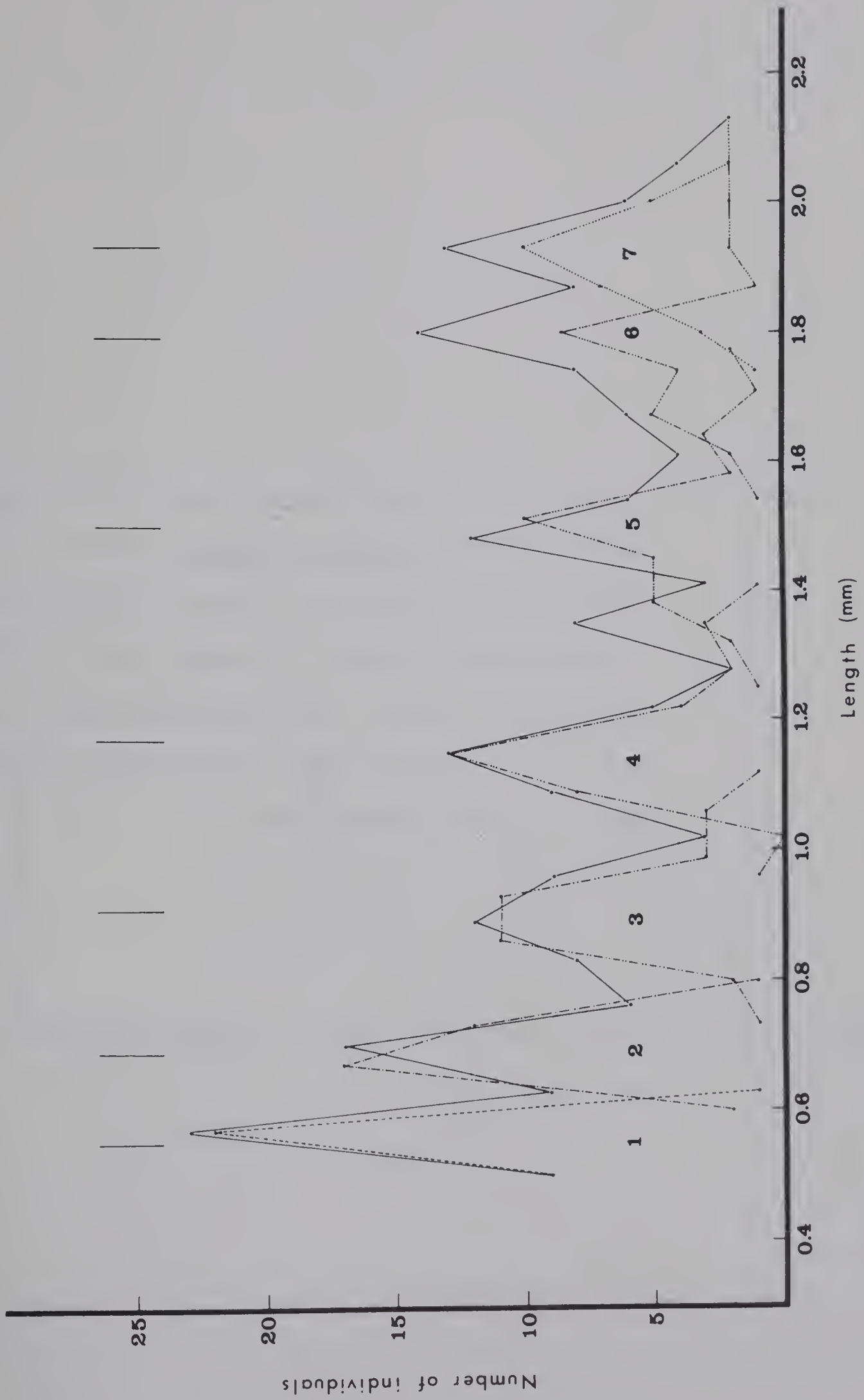
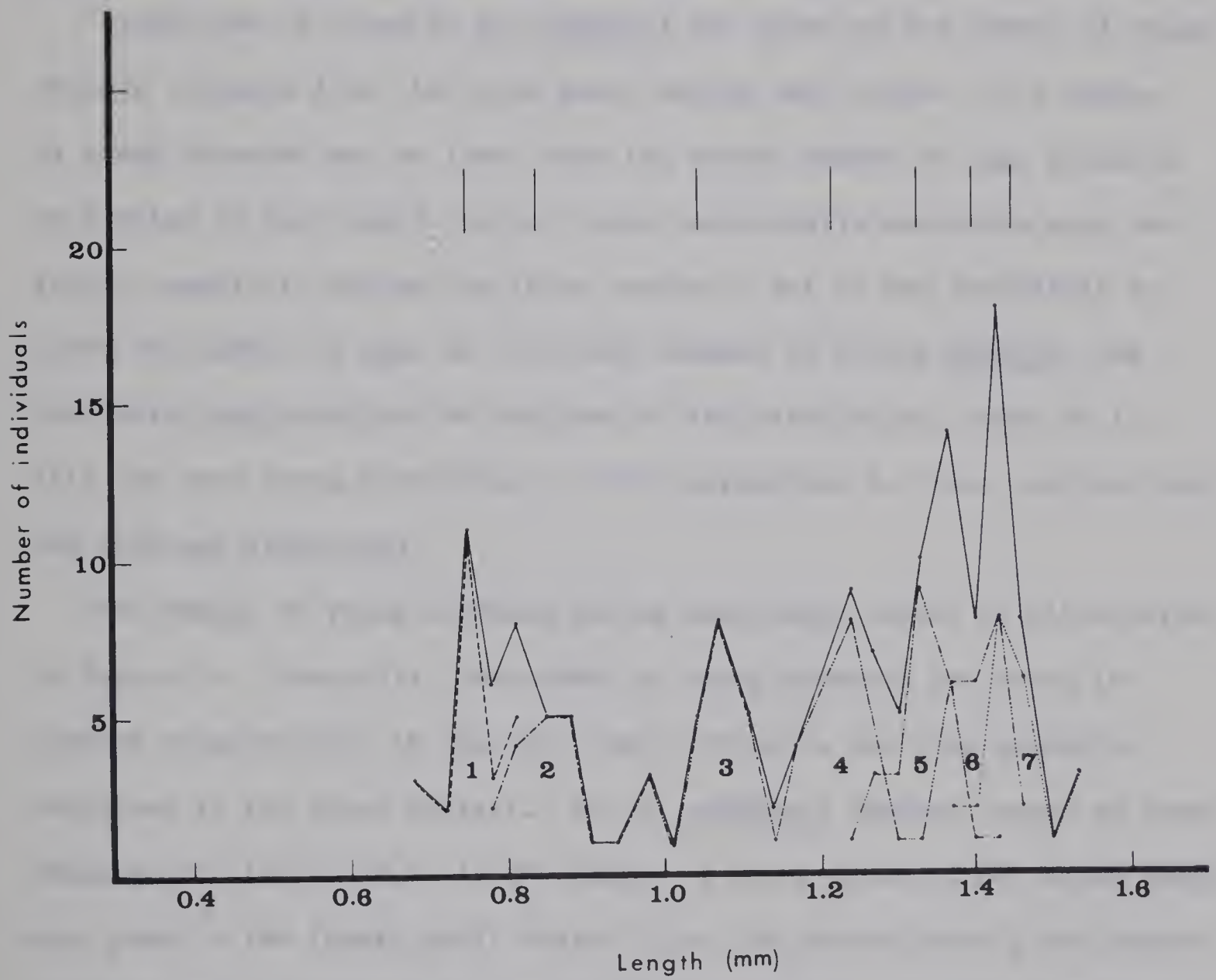


Figure 33. Size frequency distributions during the first seven instars of 17 male Daphnia schødleri reared at temperatures fluctuating from 22-29.4 C. Broken lines designate the actual individual instars. The solid line is a composite curve for all instars. The vertical bars near the upper edge of the figure represent the mean total lengths for each of the first seven instars.







various instars, and this tends to increase with instar number. Although the size-frequency distribution method may be of some use for the analysis of early instars, it is not applicable for higher instars. It does not appear to be a satisfactory method for studying growth in natural populations of Daphnia.

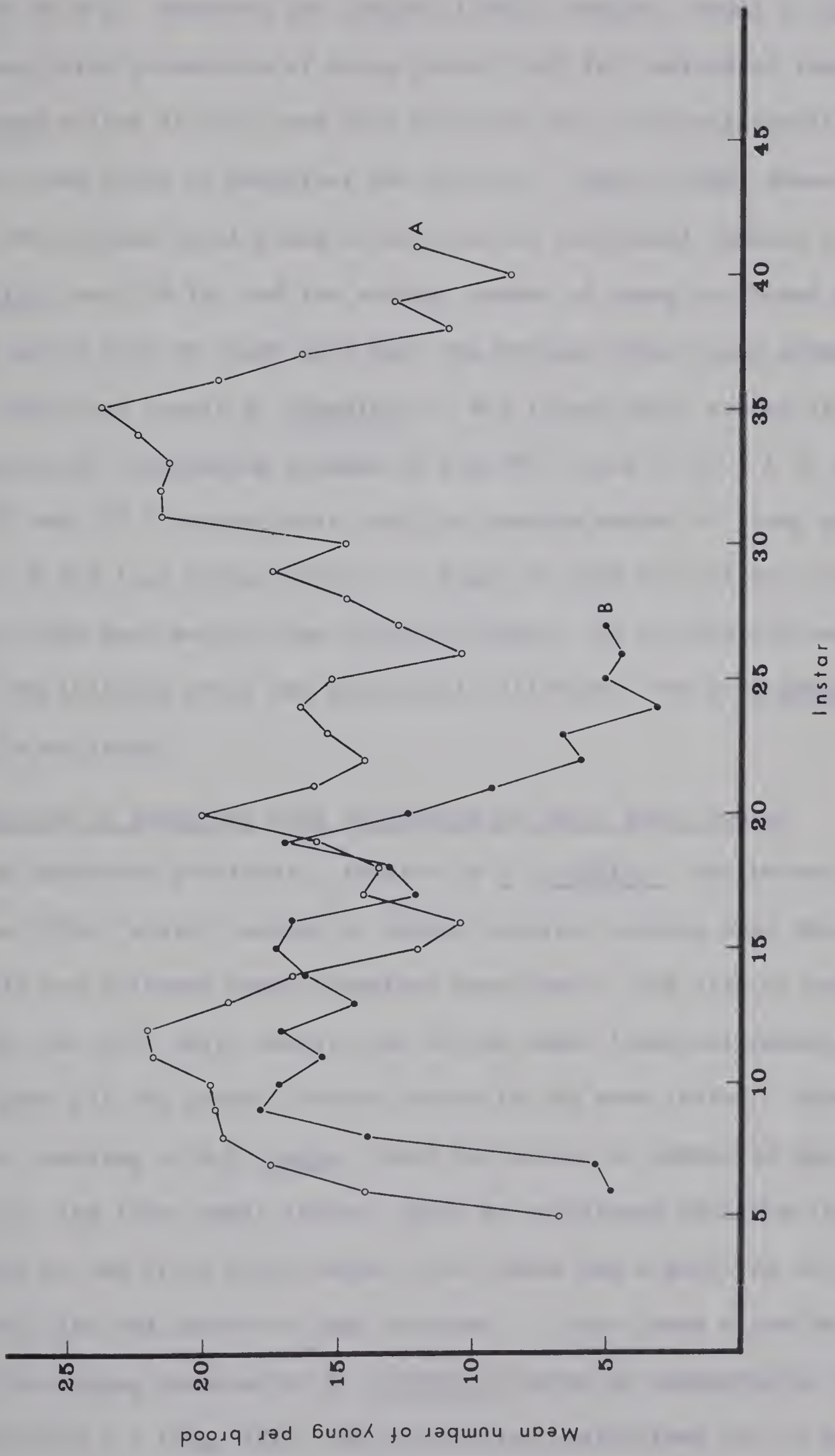
### Reproduction

#### Variation in production of young throughout life

Production of young by D. schødleri was based on the number of young animals released from the brood pouch during each instar. The number of young released may be lower than the actual number of eggs produced by females in each adult instar, since occasionally nonviable eggs were found, especially during the later instars. But it was impossible to count the number of eggs in the brood chamber of living Daphnia, and nonviable eggs could not be included in the calculation; hence it is felt the term young production is more appropriate for this section than the term egg production.

The number of young produced during each adult instar is illustrated in Figure 34. Generally, the number of young produced per brood increased progressively in the early adult instars, and then gradually decreased in the later instars. For D. schødleri females reared at room temperatures (22 to 29.4 C), the number of young produced per brood reached a peak in the fourth adult instar (i.e. the fourth brood); the number produced then fluctuated for the following ten adult instars. After the 14th adult instar, the number of young produced per brood showed a rapid decrease. For females reared at  $20 \pm 1$  C, the number of young produced per brood increased for the first eight adult instars, then decreased and fluctuated until the 22nd adult instar (i.e. the 28th instar). After the 22nd adult instar (or 22nd brood), the number of young produced per brood increased again and reached a second peak in the 31st adult instar.

- Figure 34. Mean number of young produced during each adult instar.
- A. Data based on 31 female D. schødleri primiparous in the fifth instar at  $20 \pm 1$  C.
  - B. Data based on eight female D. schødleri primiparous in the sixth instar at temperatures fluctuating between 22 and 29.4 C.





MacArthur and Baillie (1929) found that the average total production of young for individual females of D. magna was 15 at 28 C, 49 at 18 C, and 36 at 8 C. Anderson and Jenkins (1942), however, found a higher average total production of young (about 150) for individual females of D. magna raised at 25 C, and they believed their cultural conditions were better than those of MacArthur and Baillie. LeSuer (1959) showed that at 16 C the average total young production for individual females of D. schødleri was 138.16, and the average number of young per brood was 6.3. The results from my study show that the average total young production for individual female D. schødleri of Big Island Lake, reared at room temperatures fluctuating between 22 and 29.4 C and at  $20 \pm 1$  C, was 233.87 and 377.9 respectively, and the average number of young per brood was 12.5 and 16.3 respectively. It might be that the cultural conditions in my study were better than those of LeSuer. An alternative explanation would be that his stock was genetically different from D. schødleri used in my study.

#### Production of young and size of females in first adult instar

As mentioned previously, females of D. schødleri may become mature in the fifth, sixth, seventh or eighth instars, varying even when animals are cultured under identical conditions. The size of animals during the first adult instar also varies under identical conditions, even when all the animals become mature in the same instar. Green (1954), working with D. magna, found variations in number of eggs produced in the first adult instar, which he correlated with the size of females in the first adult instar, i.e. there was a positive correlation between size and number of eggs produced. I also found a similar situation for young produced by D. schødleri reared at temperatures of  $20 \pm 1$  C and 22-29.4 C (Fig. 35). The correlation coefficient for 55 females



Figure 35. Length of female and production of young in the first adult instar.

- A. Data based on 55 female D. schødleri primiparous in the fifth instar at  $20 \pm 1$  C.
- B. Data based on 27 female D. schødleri primiparous in the sixth instar at  $20 \pm 1$  C.
- C. Data based on 28 female D. schødleri primiparous either in the fifth or sixth instars at temperatures fluctuating between 22 and 29.4 C.



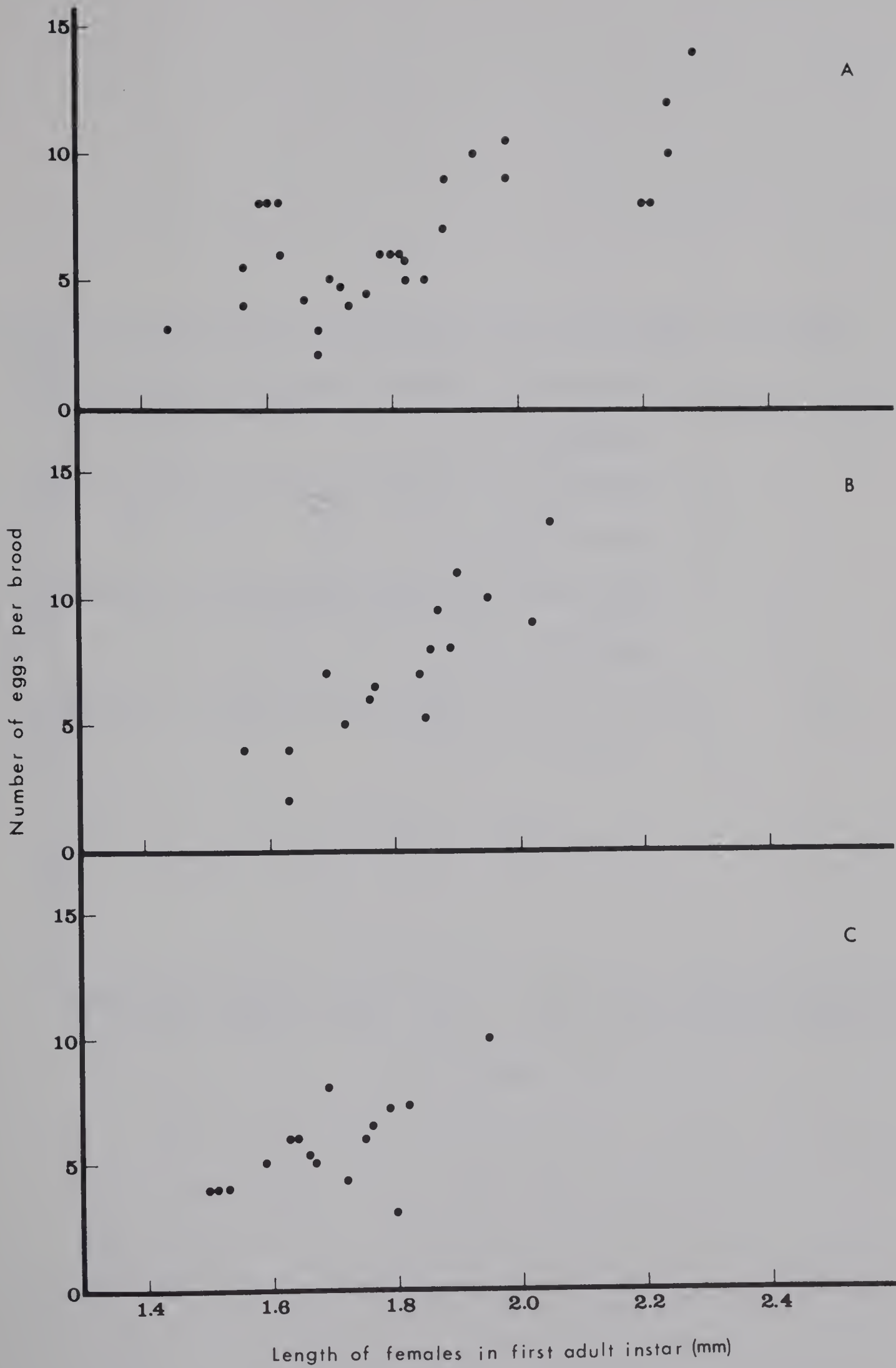
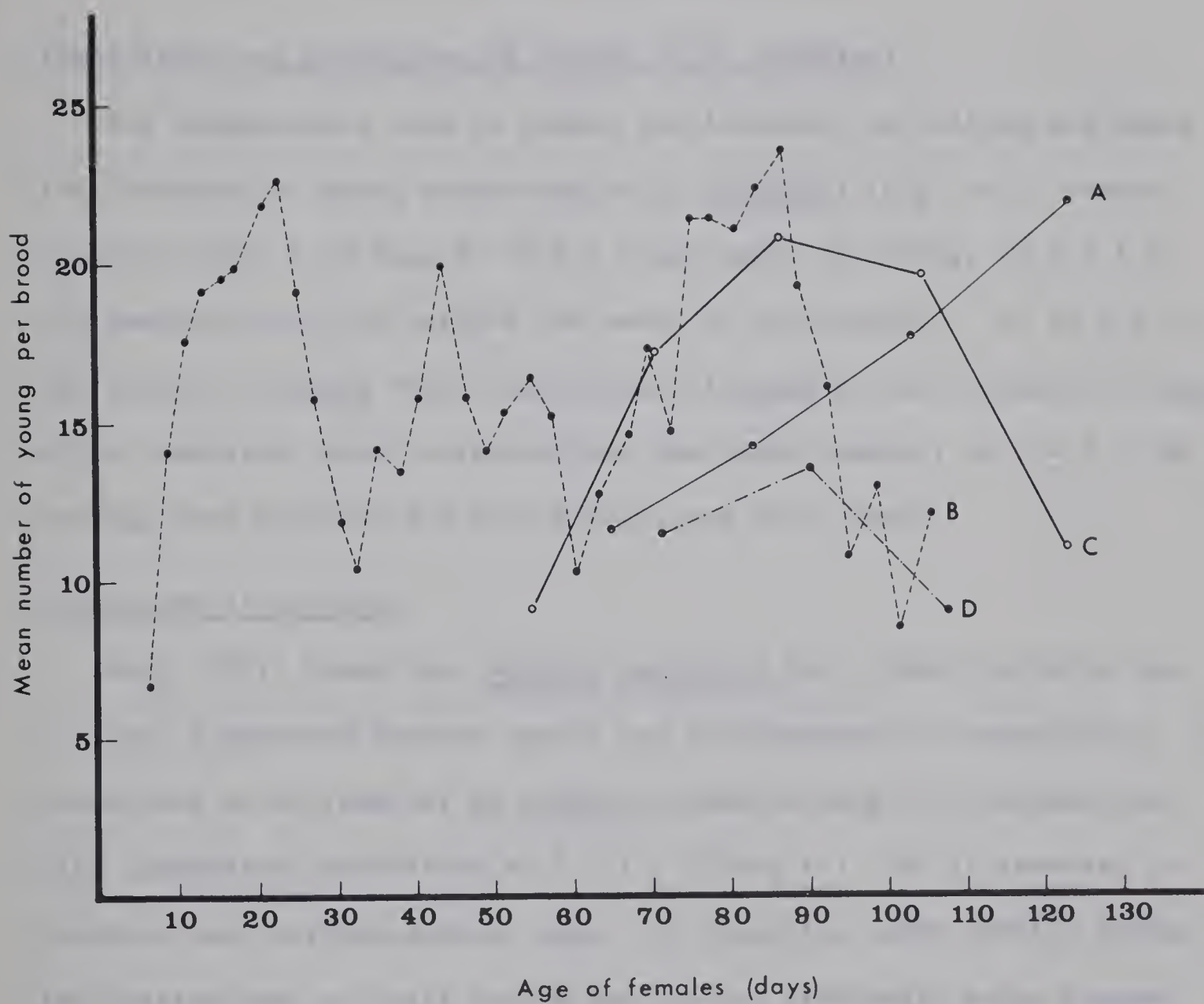


Figure 36. Mean brood size in relation to age (in days) of females.

- A. Data based on females primiparous in the seventh instar at  $5 \pm 1$  C.
- B. Data based on females primiparous in the fifth instar at  $20 \pm 1$  C.
- C. Data based on females primiparous in the sixth instar at  $5 \pm 1$  C.
- D. Data based on females primiparous in the eighth instar at  $5 \pm 1$  C.





primiparous in the fifth instar at  $20 \pm 1$  C was 0.7316 ( $P < 0.01$ ); for 27 females primiparous in the sixth instar at  $20 \pm 1$  C the correlation coefficient was 0.8659 ( $P < 0.01$ ); for 28 females primiparous in either the fifth or sixth instars at 22-29.4 C the correlation coefficient was 0.6530 ( $P < 0.01$ ). All test were highly significant; hence the number of eggs produced per female in the first brood is positively correlated with the size of females in the first adult instar.

#### Temperature and production of broods in *D. schødleri*

Low temperatures tend to reduce the frequency of molting and hence the frequency of brood production in *D. schødleri* (Fig. 36). Broods occurred every 2.72 days at  $20 \pm 1$  C and every 18.07 days at  $5 \pm 1$  C. Low temperatures also delayed the onset of reproduction. At  $20 \pm 1$  C, the females released their first brood of young on the average 6.63 days after themselves being released from the brood chamber; at  $5 \pm 1$  C the average time required for this process was 63.57 days.

#### Production of ephippia

Berg (1931) found that *Daphnia cucullata* Sars, under suitable conditions, alternated between sexual and parthenogenetic reproduction. I found this to be true for *D. schødleri* females kept in a refrigerator with temperature maintained at  $5 \pm 1$  C (Table 19). Of 21 females, 14 produced only parthenogenetic eggs, the remaining seven females producing resting eggs at least once without males previously being present. Two females produced ephippia with resting eggs after producing one brood of parthenogenetic eggs, and then they again reproduced parthenogenetically (animal number 3 and 4, Table 19); whereas one female produced an ephippium with resting eggs after producing three broods of parthenogenetic eggs (animal number 2). All females had one sterile instar (i.e. no eggs were deposited) immediately following the production of





Table 19. Reproductive characteristics of seven female Daphnia schødleri that produced at least one ephippium, at  $5 \pm 1$  C. The numbers shown in each column represent the number of young produced in the respective instar. The young produced were all females.

Animals	Instars						
	6	7	8	9	10	11	12
1	8	Eph.	0	Eph.	0	0	-
2		13	17	17	Eph.	Dead	
3		10	Eph.	0	17	-	-
4		10	Eph.	0	5	-	-
5		Eph.	0	Eph.	Dead		
6		Eph.	0	Eph.	Dead		
7		Eph.	0	Eph.	0	Eph.	0

0 indicates neither ephippial eggs nor parthenogenetic eggs was produced.

Ehp. indicates the production of an ephippium and ephippial eggs.

Dead indicates the death of the mother animal.



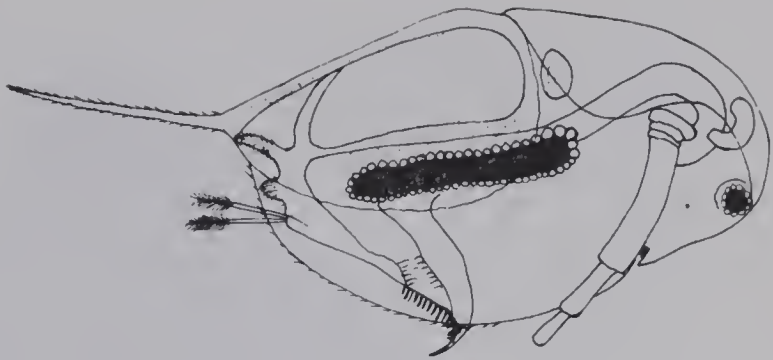
ephippia. This is contrary to observations made by Berg (1931) for D. cucullata, where the females did not have a sterile instar after producing ephippia, but instead reproduced either sexually or parthenogenetically.

Females that are to produce ephippia and resting eggs in the next instar usually can be recognized by the presence of a compact dark mass of small fat globules in the ovaries. The mass at first is very small and consists of closely packed small fat globules surrounded by larger fat globules. Near the end of the pre-ephippial instar, the compact dark mass has grown quite large, and it occupied nearly all of the ovary. The female soon undergoes ecdysis, and the new exoskeleton of female, which is now in the ephippial instar, has an indentation on the dorsal margin at the head-carapace junction. The dorsal part of carapace, which will eventually develop into the ephippium, now is light brown or gray color, and it is separated from other part of carapace by an irregular line (Fig. 37A). Very shortly two (or rarely three) fully developed resting eggs (ephippial eggs) are deposited into the modified dorsal part of carapace (Fig. 37B). The modified dorsal part of carapace now becomes darker and is gradually pushed upwards. Its separation from the remainder of the carapace becomes evident, and eventually the modified dorsal part is completely freed, by the mechanics of molting, from the rest of carapace. The female after molting and discarding the ephippium still possesses an indentation on her dorsal margin (Fig. 37D).

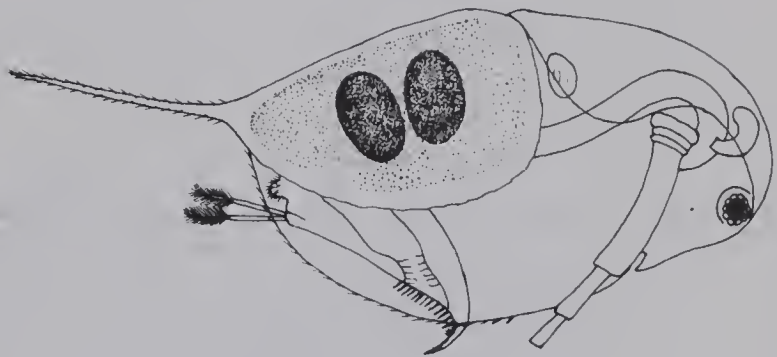
Figure 38B shows a resting egg dissected out of the ephippium (Fig. 38A). The female producing this egg was maintained at  $5 \pm 1$  C without the presence of males. This ephippium, although it was kept in fresh culture medium at  $20 \pm 1$  C for two months, failed to hatch. The appearance of resting eggs is quite different from that of parthenogenetic eggs

Figure 37. Formation of an ephippium in Daphnia schødleri.

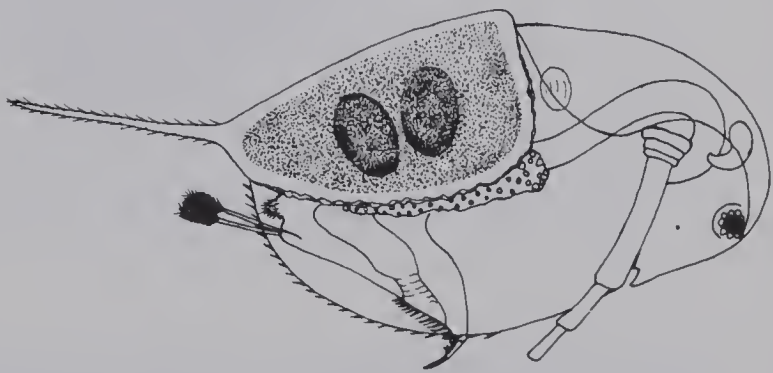
- A. Female at the beginning of ephippial instar, showing fully grown resting egg in ovary, and the modified dorsal part of carapace.
- B. Female in the middle of ephippial instar, showing two resting eggs, which have been deposited into modified dorsal part of carapace.
- C. Female near the end of the ephippial instar.
- D. Female after molting and discarding ephippium.



A



B



C



D

Figure 38. Ephippium, resting egg, and parthenogenetic egg of Daphnia schødleri.

- A. The ephippium.
- B. Resting egg dissected from an ephippium produced by a female kept in a refrigerator with temperature maintained at  $5 \pm 1$  C.
- C. A parthenogenetic egg three hours after being deposited into the brood pouch.



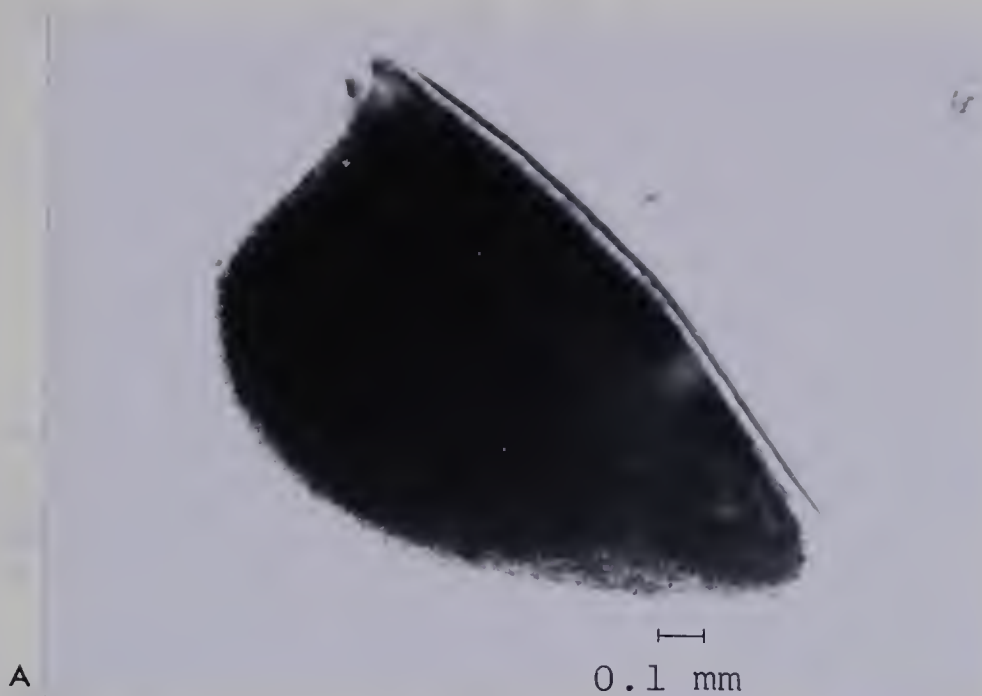




Table 20. Relationship between egg size and size of young in Daphnia schødleri.

Animals	Number of eggs mea- sured	Number of young mea- sured	Mean egg volume $\text{mm}^3 \times 10^3$	Mean length of young mm
1	13	13	6.1749	0.6392
2	12	11	5.5403	0.6308
3	9	5	5.4228	0.5987
4	12	12	7.6716	0.6830
5	7	5	5.6908	0.6175
6	10	9	8.4395	0.7027
7	10	9	6.0056	0.6146
8	6	6	11.0117	0.7713
9	14	13	7.4403	0.6770
10	10	6	5.1383	0.5628



(Fig. 38C). The parthenogenetic eggs usually consist of coarser fat (yolk) globules with one large yellowish fat globule in the center of egg; whereas resting eggs lack the large yellowish fat globule, consisting entirely of small fat globules.

#### Relation Between Egg Size and Size of Young

For Daphnia, as in other Cladocera that do not secrete a nutritive fluid for their embryos, the size of young are usually considered proportional to the size of the eggs. To test this relationship for D. schødleri, eggs were dissected from 10 females, the diameters of the eggs were measured, and the eggs were then left to develop in filtered aquarium water. Two days later active young had developed from these eggs. The length of these young were measured, and the results are given in Table 20, indicating that large eggs do tend to give rise to larger young. The calculated coefficient of correlation ( $r$ ) was 0.9696 and is significant ( $P < 0.01$ ).

#### Size of First Adult Instar Females and Size of Their Young

To determine whether there is a relationship between the size of females in the first adult instar and the size of young produced by these females, the sizes of young were plotted against the sizes of their mothers (Fig. 39). The results show that there is a positive correlation ( $r = 0.6854$ ,  $P < 0.01$ ) between the size of females in the first adult instar and the size of young produced by these females.

Agar (1914) has shown for a monoclonal population of Simocephalus exspinosus (Koch) having females of the same age and size that the size of the eggs (as estimated by the size of the young developed from them)

Figure 39. Relationship between the size of young and the size of first adult instar females producing these young.



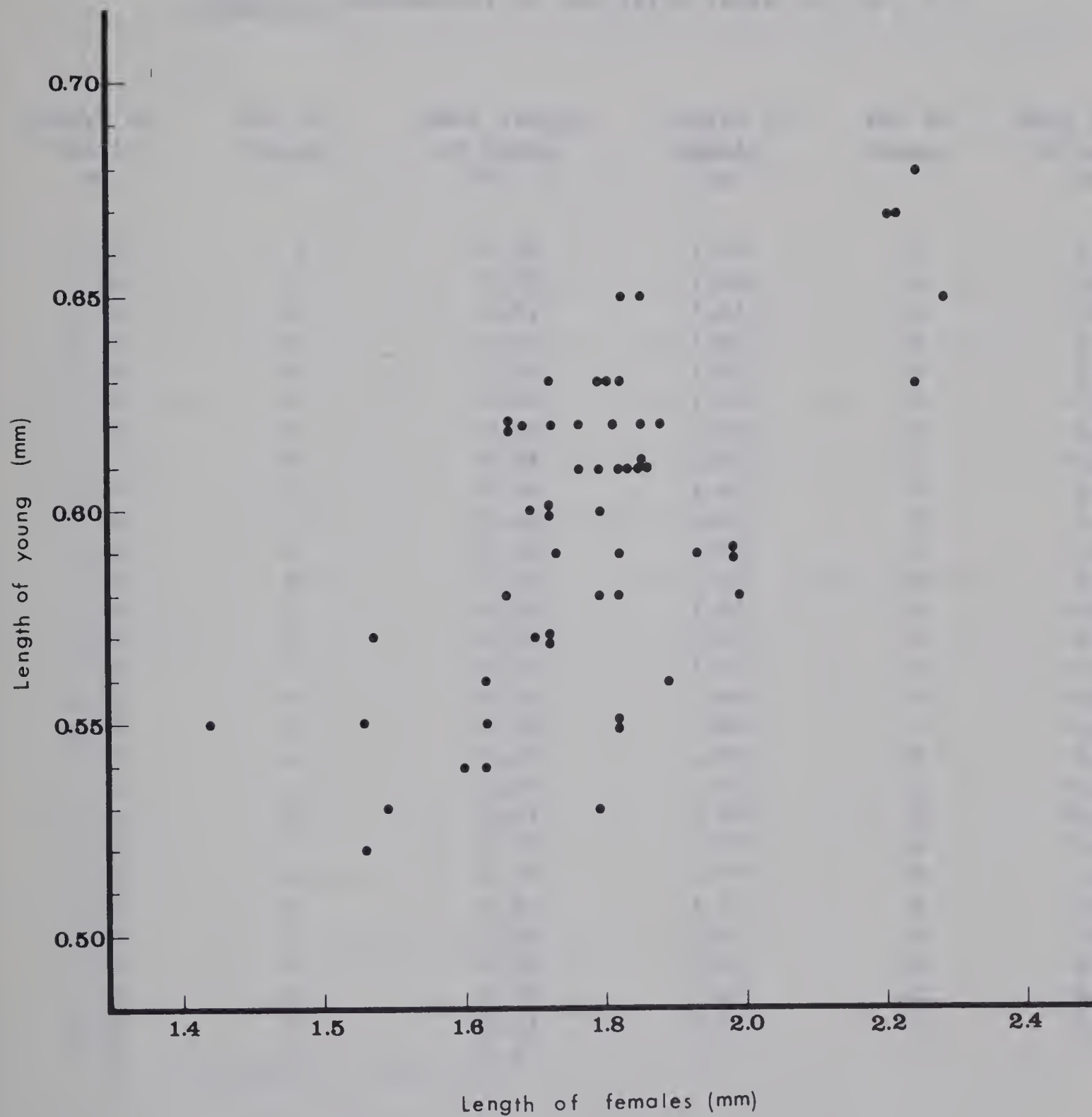




Table 21. Relationship between size of female in the first adult instar and the size of their young. Data based on 55 female Daphnia schødleri primiparous in the fifth instar at 20 + 1 C.

Length of female mm	No. of young	Mean length of young mm	Length of female mm	No. of young	Mean length of young mm
1.44	3	0.55	1.79	8	0.58
1.56	3	0.55	1.80	4	0.63
1.56	8	0.52	1.81	6	0.62
1.57	4	0.57	1.82	3	0.63
1.59	8	0.53	1.82	4	0.61
1.60	8	0.54	1.82	4	0.65
1.63	6	0.56	1.82	5	0.59
1.63	6	0.55	1.82	7	0.55
1.63	8	0.54	1.82	7	0.58
1.66	3	0.62	1.82	9	0.55
1.66	4	0.62	1.83	5	0.61
1.66	6	0.58	1.85	4	0.65
1.68	2	0.62	1.85	4	0.61
1.69	3	0.60	1.85	5	0.61
1.70	5	0.57	1.85	6	0.62
1.72	4	0.63	1.85	6	0.61
1.72	4	0.60	1.88	7	0.62
1.72	4	0.60	1.89	9	0.56
1.72	4	0.57	1.93	10	0.59
1.72	5	0.62	1.98	5	0.59
1.72	7	0.57	1.98	14	0.59
1.73	4	0.59	1.99	9	0.58
1.76	4	0.61	2.20	8	0.67
1.76	5	0.62	2.21	8	0.67
1.79	4	0.63	2.24	10	0.68
1.79	5	0.60	2.24	12	0.63
1.79	5	0.53	2.28	14	0.65
1.79	7	0.61	-	-	-



varies inversely with the number of eggs produced. For specimens producing the same number of eggs, the size of eggs varies with the size of the animal. This seems also to be the case for D. schødleri (Table 21); however these relationship apply only to eggs laid by females of the same age, since it has been shown by Green (1954), and also will be shown later in this study, that the size of the eggs and young varies with the age of the mother.

#### Relationship between Age of Females and the Size of Their Young

A total of 1,306 young D. schødleri were measured during their first instar (i.e. when first liberated from their mother's brood chamber). They varied in size from 0.46 mm to 0.78 mm, with a mean of 0.60 mm. Mean size of young tended to increase with the mother's age, at least in the early broods (Fig. 40 & 41, and Table 22).

Figure 40 is based on data from one female D. schødleri that was primiparous in the fifth instar, and which survived for 34 instars at  $20 \pm 1$  C. For this female the mean size of young increased in the early broods, and then decreased in later broods. Figure 41 and Table 22 are based on six females, and here also there are indications that the mean size of young liberated tend to increase with the age of the mother.

#### Temperature and the Size of Young Produced

For Simocephalus vetulus, Agar (1913) found that the size of young produced at lower temperatures is larger than the size of young produced at higher temperatures. To determine if this relationship existed for D. schødleri, the sizes of young produced by females at  $20 \pm 1$  C were compared with those produced at  $5 \pm 1$  C (Table 23). The mean length of young produced by D. schødleri females was 0.56 mm at  $20 \pm 1$  C and 0.65 mm

Figure 40. Influence of maternal age upon size of young. Data are based on one female Daphnia schødleri that was primiparous in the fifth instar and survived for 24 instars at  $20 \pm 1$  C.



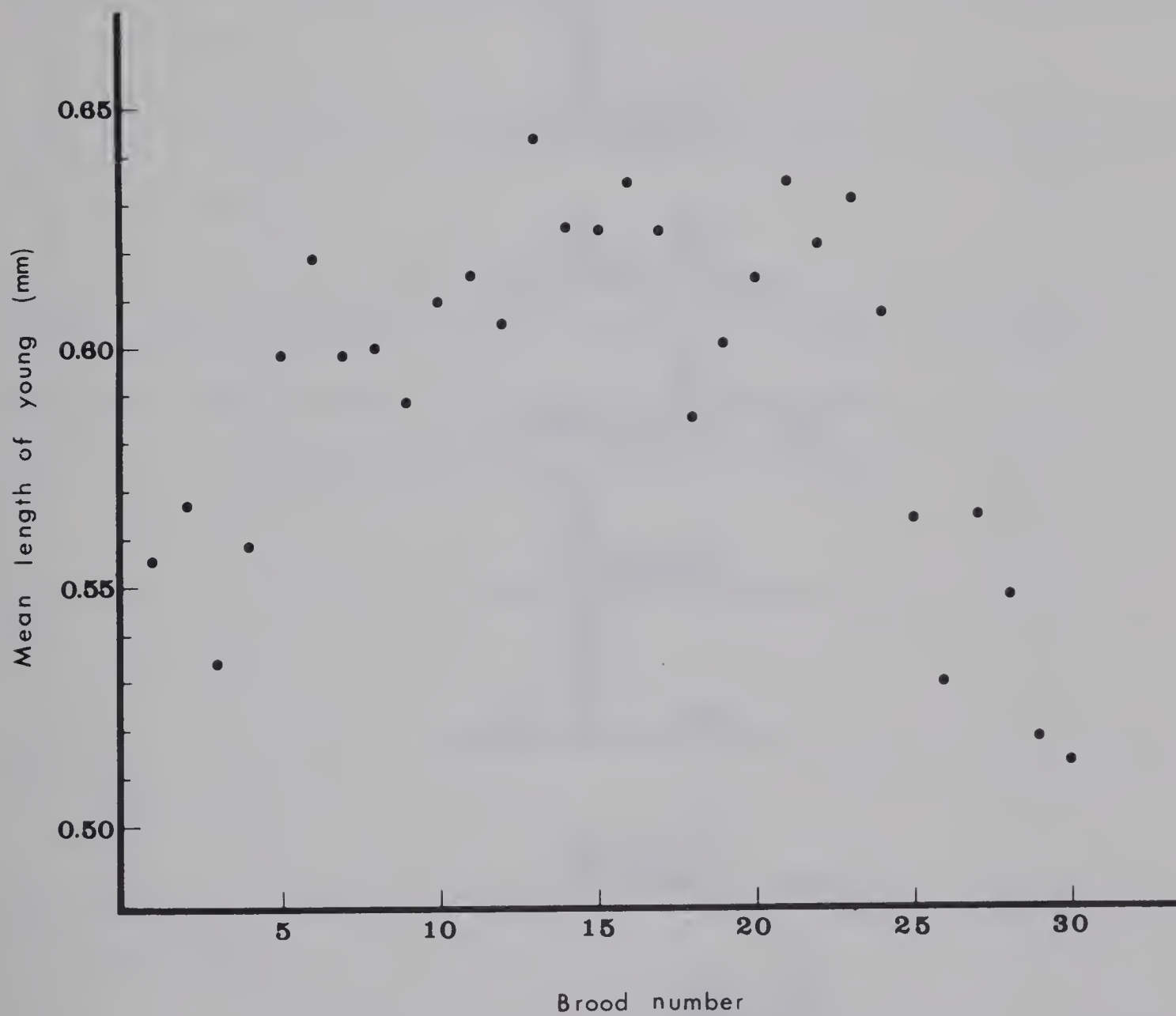


Figure 41. Length of liberated young in different broods, shown as percentage of size frequency distribution. The arrows indicate the mean length of the young.

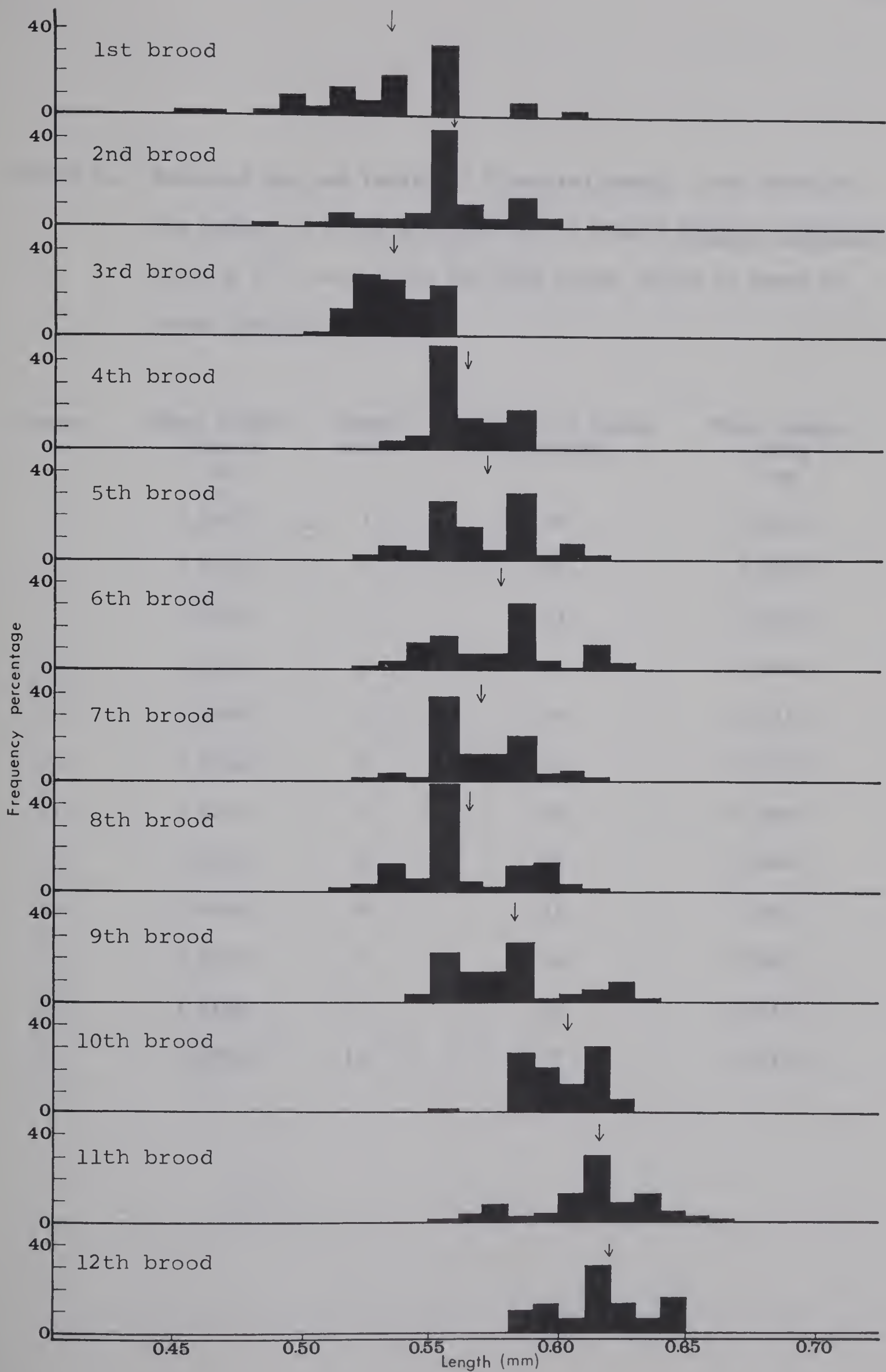




Table 22. Maternal age and length of liberated young. Data based on the number of young produced by six female Daphnia schødleri at  $20 \pm 1$  C, except for the 12th brood, which is based on three females.

Instar	Mean length female mm	Brood number	Number of young measured	Mean length young mm
5	1.6651	1	44	0.5346
6	1.9256	2	62	0.5590
7	2.0096	3	63	0.5359
8	2.1483	4	66	0.5646
9	2.2669	5	66	0.5715
10	2.3346	6	66	0.5778
11	2.4245	7	66	0.5693
12	2.5014	8	66	0.5642
13	2.6146	9	67	0.5820
14	2.7040	10	66	0.6031
15	2.7186	11	62	0.6151
16	2.7083	12	29	0.6192





Table 23. Temperature and size of young liberated.

20 ± 1 C				5 ± 1 C		
Maternal instar no.	Mean length female mm	No. of young	Mean length young mm	Mean length female mm	No. of young	Mean length young mm
6	1.9256	62	0.5590	2.0995	46	0.6326
7	2.0096	63	0.5359	2.3507	52	0.6392
8	2.1483	66	0.5646	2.5458	62	0.6502
9	2.2669	66	0.5715	2.6790	59	0.6457
10	2.3346	66	0.5770	2.8275	11	0.6958



at  $5 \pm 1$  C, indicating that the young produced by D. schødleri females at lower temperature are indeed larger than those produced at higher temperature.

### Development of Parthenogenetic Eggs

#### in vitro

Obreshkove and Fraser (1940), for D. magna, successfully studied the development of parthenogenetic eggs in vitro; but, to my knowledge, this method has not been used to study other species of Daphnia. In my study, the development of parthenogenetic eggs of D. schødleri was observed in vitro at 18 C. Eggs, three hours after being deposited into brood pouch, were dissected out of the female under a dissecting microscope, using fine dissecting needles. The eggs were then transferred to a depression slide filled with filtered aquarium water. Several series of eggs developed in vitro using this method.

The parthenogenetic eggs of Daphnia are enclosed in two egg membranes, i.e. a thin inner membrane called the vitelline membrane, and a thicker outer membrane, which was called a chorion by Lebedinsky (1891). Obreshkove and Fraser (1940), in their in vitro studies of D. magna eggs, found that the two membranes became progressively resistant to mechanical injury after the deposition of the eggs; three hours after being deposited these eggs could withstand a centrifugal force of 1,800 times gravity for several minutes without disintegration. Accordingly, the three-hour-old eggs of D. schødleri were chosen as the initial development stage for my study.

Eggs at three hours appear yellowish-green, and they are opaque. With lapse of time, the eggs become light green and translucent, and the periphery becomes progressively clearer (Fig. 42-1'). At the 6-hour stage

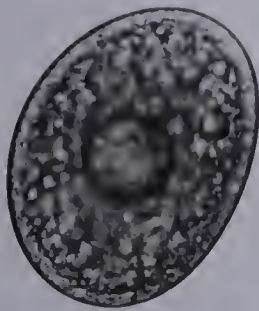
Figure 42. Development of Daphnia schodleri eggs and embryos in vitro. All pictures were taken under a compound microscope at a magnification of 100.

- 1'. Egg 3 hours after deposition.
- 2'. Egg at 6 hours, with a transparent periphery.
- 3'. Egg at 9 hours, showing at upper-right surface the first invagination.
- 4'. Egg at 12 hours, showing the first demarcation of the future cephalic region. A constriction (a transparent spot) at the extreme posterior end marks the beginning of the bilaterally symmetrical plane of development.
- 5'. Egg at 13 hours.
- 6'. Egg at 15 hours, showing a clear constriction at the posterior end, and further demarcation of the cephalic portion.
- 7'. Egg at 18 hours, showing further demarcation of the cephalic and abdominal appendages.
- 8'. Embryo at 21 hours, showing further demarcation of the cephalic and abdominal appendages and the more prominent bilaterally symmetrical development. The embryo has just cast off its outer egg membrane (chorion), but it is still enclosed in the inner (vitelline) egg membrane. The cast outer egg membrane is at the side of the embryo. A blastodermic thickening begins to appear at the cephalic region, giving the first external evidence of brain development.
- 9'. Embryo at 22 hours (dorsal view), showing further demarcation of abdominal appendages and development of prospective brain. The antennae appear as a pair of short, forked stubs, closely appressed to the body. The thick mid-dorsal longitudinal fold has begun to thin out laterally and posteriorly to form the carapace.
- 10'. Embryo at 24 hours (dorsal view), showing further development of brain mass, carapace and other structures.
- 11'. Embryo at 24 hours (ventral view), showing the formation of labrum and mandibles. The labrum appears as a round lobe at the cephalic region; and two mandibles appear as two smaller lobes, one on each side of prospective labrum.
- 12'. Embryo at 27 hours (side view), showing the abdominal appendages appearing as stubby blocks of tissue. Brain mass can be seen clearly in the cephalic region. A grayish mass of granular substance appears antero-dorsally to the prospective brain mass.

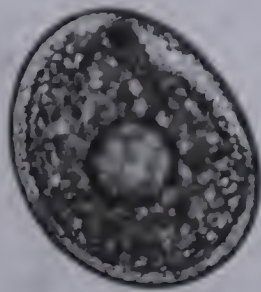




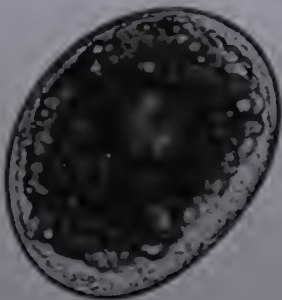
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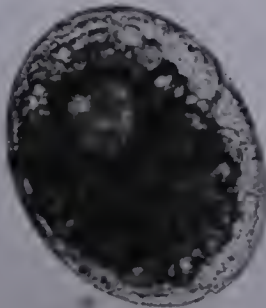
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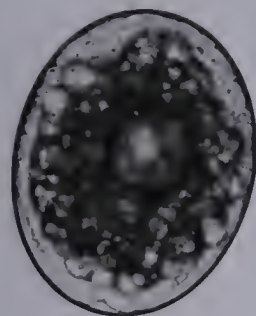
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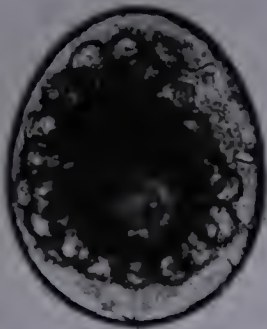
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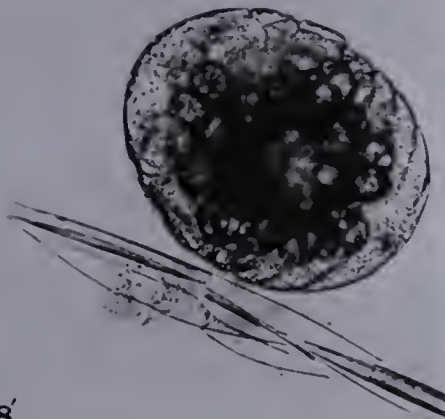
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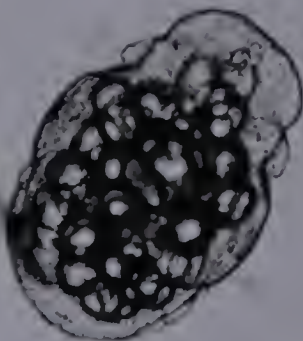
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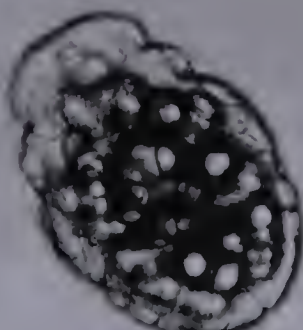
8'



9'



10'



11'

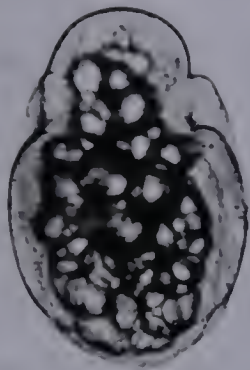


12'

Figure 42. (Continued)

- 13'. Embryo at 27 hours (dorsal view), showing a definite head bulge, and further developmental differentiations.
- 14'. Embryo at 30 hours (dorsal view), showing further development of carapace; the caudal spine has now appeared at the posterior end of carapace. Joints of antennal rami are also distinguishable but not in focus.
- 15'. Side view of embryo at 30 hours.
- 16'. Embryo at 33 hours (dorsal view), showing the appearance of the two small pink eyes; and posteriorly, the ocellus (the black body in front of the brain mass).
- 17'. Embryo at 33 hours (side view). The ocellus appears as a small pigment body ventral to the brain mass, and two eyes are located just antero-dorsal to the brain mass, although difficult to locate in this picture.
- 18'. Embryo at 36 hours (dorsal view), showing two distinct brown eyes.
- 19'. Side view of an embryo at 38 hours, showing an infolding of the cephalothorax. The carapace now covers nearly all parts of body. The caudal spine is curled ventrad and anteriorly.
- 20'. Embryo at 39 hours (dorsal view), showing further differentiation of the two eyes.
- 21'. Side view of an embryo at 39 hours. Embryo is still enclosed in the inner egg membrane. Intestine and hepatic caecum are visible.
- 22'. Embryo at 42 hours (ventral view), after casting its inner egg membrane (vitelline). The two eyes are nearly fused. Distal portion of antennae have completely separated from the body.
- 23'. Side view of embryo at 43 hours, showing the fused compound eye. The curled caudal spine adheres closely to the postabdomen. The three terminal antennal setae are much shorter than the ramus.
- 24'. Side view of embryo at 45 hours.

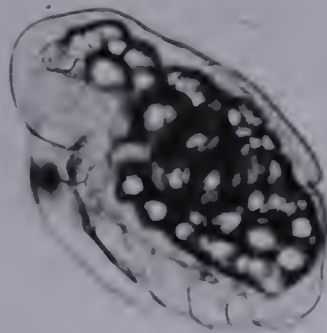




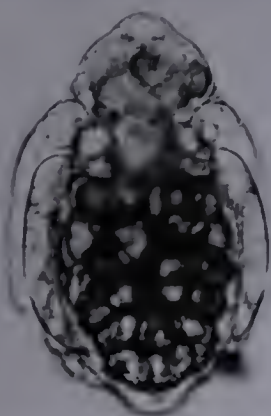
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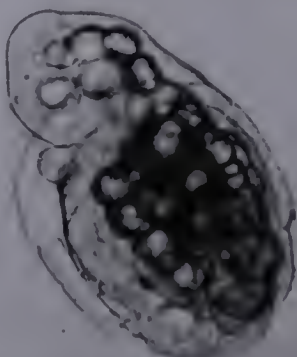
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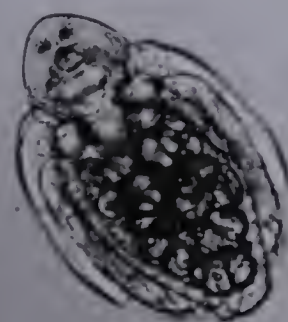
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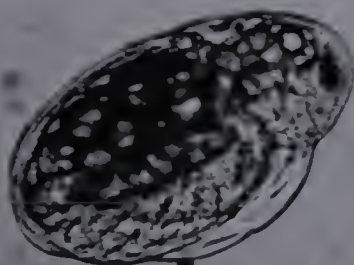
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17'



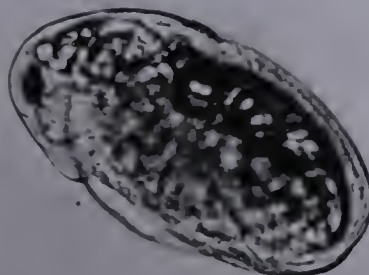
18'



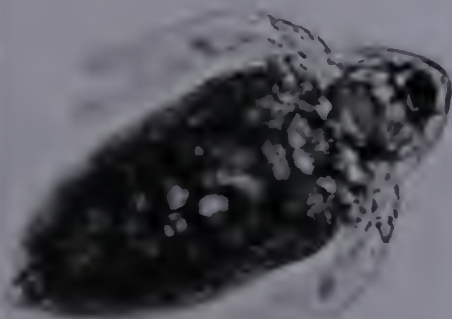
19'



20'



21'



22'



23'



24'

Figure 42. (Continued)

- 25'. Dorsal view of embryo at 45 hours.
- 26'. Side view of embryo at 48 hours.
- 27'. Ventral view of embryo at 48 hours.
- 28'. Side view of embryo at 51 hours.
- 29'. Latero-ventral view of embryo at 53 hours.
- 30'. Embryo at 57 hours, representing a fully developed young. The caudal spine has extended, and the three terminal antennal setae have become longer than the ramus.







(Fig. 42-2') a transparent edge can be seen. The edge is somewhat granular, indicating the cells are forming near the surface. At the 9 hour stage (Fig. 42-3') an invagination, which later separates the second antennae from the body proper, appears near the future anterior end of embryo. This is the first morphological change observed in the course of the in vitro development. During the succeeding 3 hours, the invagination becomes more distinct, and a vertical constriction appears at the extreme posterior end of the egg at 12 hours (Fig. 42-4'), marking the future bilaterally symmetrical plan of the organism. Eggs from the 3 hour to 18 hour stage did not show a significant increase in size.

At 18 hours the abdominal appendages appear. The body's segmentation becomes more prominent at 21 hour stage, and the prospective left and right halves of the body also are evident. At this stage the embryo, having just cast off its outer egg membrane (chorion), showed a slight increase in size. The second antennae are present as short, forked stubs, closely appressed to the body. Also a blastodermic thickening appears in the cephalic region, indicating the first external evidence of brain development. At 22 hours the future brain of the embryo is distinguishable. A mid-dorsal longitudinal fold has begun to thin out laterally and posteriorly to form the carapace. At 24 hours the head bulge becomes distinguishable, and the second antennae increase in length. A granular mass which will subsequently differentiate into the eye, appears antero-dorsad to the prospective brain at 27 hours. This granular material, which at first is present as a single mass, grayish in color, differentiates into two very small pink bodies by 30 hours; but they are very small at this time and difficult to demonstrate. However, these pink bodies can be easily distinguished in 33-hour embryos. At this stage, the prospective ocellus appears as a dense mass.





At 30-hours the dorsal spine is clearly visible, extending out from the posterior end of prospective carapace. With subsequent development, this spine will continue to increase in length and eventually will curl ventrad and anteriorly between the posterior edges of the carapace. The joints of the second antennae first become visible in the 27-hour embryo, with the three terminal setae of the second antennae becoming distinguishable in the 36-hour embryo. A few feeble and irregular heart beats were observed in the embryos of 30 hours. Slow lateral expansions and contractions of the entire body, indicating the first sign of movement of the body, were also occasionally observed in 30-hour embryos. The embryo, although casting off its outer egg membrane (chorion) at the 21 hour stage, remained enclosed in the inner egg membrane until the 39 hour stage. This membrane tended to restrict the movement of the embryo. After the 39 hour stage, the embryo now being free of its inner egg membrane, greatly increased in body length. After the 39 hour stage the embryo showed vigorous movements, and at this time it was capable of moving about by moving the second antennae. Intestine and hepatic caeca were clearly visible at 39 hours.

At 42 hours the distal portion of the second antennae separated from the body; and the two rami, originally appressed to each other, also separated. The two small pink eyes gradually increase in size, and eventually become completely fused at 45 hours. From 42 hours until 56 hours, no significant changes in external morphology were observed. During the 57 hour stage, the caudal spine begins to extend, the embryo rapidly increases in size, and the three terminal setae of the second antennae become longer than their rami. After the extension of the caudal spine the individuals resemble in every respect young just released from the brood chamber of the mother. Thus, they complete their embryonic development from eggs to independent free-swimming organisms in about 57 hours at 18 C.



Development of Parthenogenetic Eggs in  
the Brood Chambers of Females

General observations

Parthenogenetic eggs were observed being deposited into brood chamber on 246 occasions. The parthenogenetic eggs were deposited into the brood chamber from 2 to 49 minutes after molting at a temperature of  $20 \pm 1$  C, the average time of deposition being 14 minutes after molting. On only 7 occasions were the eggs deposited into the brood chamber 30 minutes or more after molting. As previously mentioned, the oviducts are located on the dorsal and posterior end of the ovaries, and they lead into the brood chamber. The oviducts are very narrow, and during the process of egg laying, the eggs are squeezed out through a narrow channel of the oviduct as elongate structures. In the brood chamber, the newly laid eggs, during the first three hours of development, start to swell and obtain a more ovoid shape. In the early stages the eggs are dark grayish or grayish green, and the egg content consists of yolk globules of various sizes, with a large yellowish oil globule in the center. With succeeding development, the eggs become yellowish-green, and the periphery of the eggs gradually becomes transparent and somewhat granular. Subsequent developmental processes are the same as those observed in vitro.

When the young were released from their mother's brood chamber, they usually had attained the development similar to that of 53 hour stage in vitro (see Fig. 42-29'). The young are released from the brood chamber by the ventral flexion of the postabdomen of the females. When released the caudal spine is still curled, and the three terminal antennal setae are still shorter than their rami. Most of the young extend their caudal spines and increase in body size soon after being released from the brood chamber. Also the three terminal antennal setae very shortly become longer





than their rami. Occasionally, due to external stimulations, such as a stimulus caused by handling during the course of observation, some embryos at a stage resembling the 42 hours embryo in vitro (Fig. 42-22') were released from their mother's brood chambers. These embryos were not completely developed and they spend considerable time, usually several hours, swimming about in the culture medium before development was completed.

For the study of embryonic development in the brood chamber, eight easily recognizable stages of embryonic development, similar to those proposed by Green (1956), were selected and defined as follows:

- Stage I        Eggs opaque or translucent with transparent edges. At first, eggs are dark grayish or grayish-green; but later they become yellowish-green, and a clear zone begins to form around the periphery of the egg (Fig. 42-1' & 2').
- Stage II       Eggs with markedly granular transparent edges and an invagination giving the first indication of the prospective cephalic region. Later the invagination becomes more prominent, and a constriction appears at the extreme posterior end (Fig. 42-3' to 7').
- Stage III      Embryo apparent, but head not yet defined; outer egg membrane (chorion) cast off; segmentation of body prominent. In latter part of stage, the antennae are present as short, forked stubs closely appressed to the body; and the thick mid-dorsal longitudinal fold begins to grow out forming the carapace (Fig. 42-8' to 11').





- Stage IV      Embryo with distinct head bulge; antennae longer but still closely appressed to the body; abdominal appendages appear as stubby blocks of tissue (Fig. 42-12' to 15').
- Stage V        Embryo with two small pink eyes and longer antennae, the latter still appressed to the body, and with joints of rami clear (Fig. 42-16' & 17').
- Stage VI       Embryo with two distinct brown eyes; three terminal antennal setae visible but much shorter than their rami; body completely enclosed in carapace (Fig. 42-18' to 21').
- Stage VII      Embryo with two large black eyes very close to each other; inner egg membrane (vitelline) cast off; distal portion of antennae separated from body (Fig. 42-22').
- Stage VIII     Embryo with one large black eye (Fig. 42-23' to 29').

#### Duration of embryonic stages in brood chamber

Approximate duration of each embryonic stage was determined for animals kept at temperatures of  $20 \pm 1$  C and  $5 \pm 1$  C (Table 24). Data for females kept at  $20 \pm 1$  C were based on 10 broods; and for females kept at  $5 \pm 1$  C data were based on 46 broods. Low temperature increased the duration of each embryonic stage, and hence the entire embryonic period. However, the increase in the duration of each embryonic stage caused by a decrease in temperature was not necessary in the same proportion as the decrease in temperature.

#### Frequency distribution of embryonic stages in natural populations

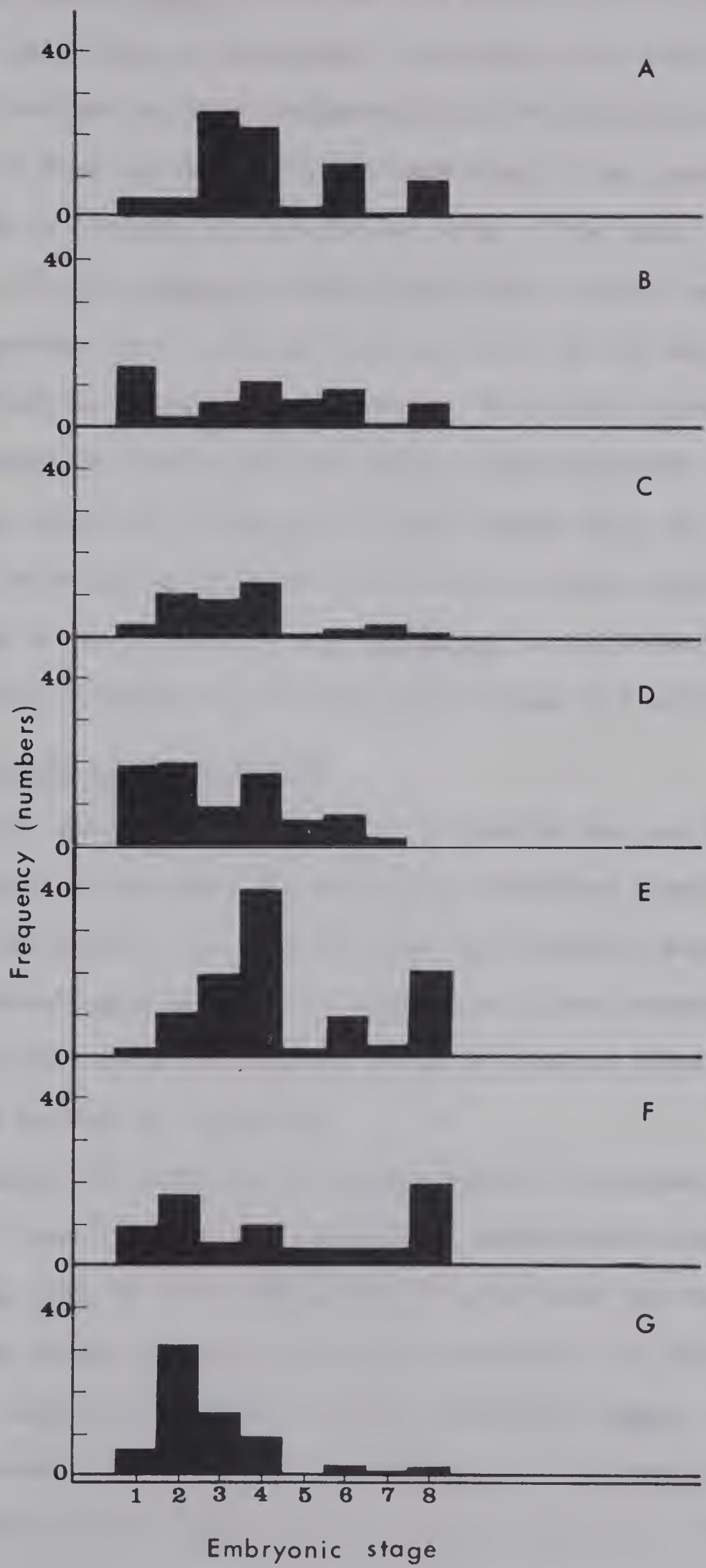
As shown in Table 24, the eight embryonic stages of D. schødleri are not of the same duration. However Green (1956), on several occasions,



Table 24. The duration of embryonic stages of Daphnia schødleri in the brood chamber.

Stage	Mean duration (hrs)	
	5 $\pm$ 1 C	20 $\pm$ 1 C
1	64.08	10.09
2	71.04	8.14
3	45.84	6.05
4	64.08	6.92
5	24.00	3.19
6	42.72	4.66
7	24.00	2.95
8	65.52	11.28

- Figure 43. Frequencies of embryonic stages in samples from natural population of Daphnia schødleri in Big Island Lake.
- A. 80 females collected on 13 July 1966.
  - B. 57 females collected on 19 July 1966.
  - C. 41 females collected on 27 July 1966.
  - D. 77 females collected on 27 May 1967.
  - E. 105 females collected on 13 June 1967.
  - F. 69 females collected on 24 June 1967.
  - G. 64 females collected on 3 July 1967.







noted that female Daphnia collected from ponds were carrying embryos in about the same stage of development, concluding that either the eggs might be laid more or less synchronously by the females in natural populations, or that the females in the same stage of any reproductive instar might tend to congregate in particular areas of the pond. To test this hypothesis for D. schødleri in Big Island Lake, several samples of females carrying embryos were collected from the lake, and the embryonic stages were analyzed by frequency distribution. The results showed that at certain times the females were carrying a large number of embryos of a particular stage and few embryos of other stages (Fig. 43). The evidence, although inconclusive, gives some indication of either synchronization in egg laying in the population of D. schødleri in Big Island Lake, or of congregation of females in the same instar stage at a particular place.

#### Duration of the brooding periods

Anderson and Jenkins (1942) first introduced the term brooding period to designate the time that the developing individual spends in the brood pouch of the mother, i.e. from the time the individual passes as an egg into the brood pouch until it is released as a free swimming young. The fully developed young are normally released from the brood pouch shortly before the molting of the mother.

I observed the duration of brooding periods for animals at temperatures of  $20 \pm 1$  C and  $5 \pm 1$  C. The results are summarized in Tables 25 and 26. The average time of the brooding period varied with the length of the instar of the female carrying the brood in question (see Tables 16, 17, 25 and 26). Anderson and Jenkins (1942), studying D. magna, showed that successive adult instars tended to increase in time, and the brooding period increased with this increase in instar duration. This is also



Table 25. Duration of the brooding period for female Daphnia schødleri primiparous in the fifth instar at  $20 \pm 1$  C.

Instar	Mean duration of brooding period hours	Number of broods observed
5	51.79	26
6	51.51	24
7	50.76	24
8	50.25	24
9	52.98	24
10	51.75	24
11	53.15	24
12	53.96	24
13	53.23	24
14	54.80	22
15	55.77	22
16	54.86	20
17	55.96	20
18	55.36	19
19	55.01	20
20	55.28	19
21	56.47	18
22	55.69	16
23	55.91	12
24	56.41	13
25	56.89	9
26	56.31	12
27	56.18	13
28	54.80	13
29	55.22	14
30	54.49	9
31	55.49	11
32	54.93	6
33	53.93	6
34	52.06	3
35	52.38	2
36	54.45	6
37	54.81	5
38	56.55	5
39	55.32	4
40	55.08	2



Table 26. Duration of the brooding period in days for female Daphnia schødléri primiparous in sixth, seventh and eighth instar at  $5 \pm 1$  C.

Instar	Sixth instar			Seventh instar			Eighth instar		
	Mean duration of brooding period days	Number of broods ob-served	Mean duration of brooding period days	Number of broods ob-served	Mean duration of brooding period days	Number of broods ob-served	Mean duration of brooding period days	Number of broods ob-served	Mean duration of brooding period days
6	12.8	5	-	-	-	-	-	-	-
7	15.3	3	15.9	10	-	-	-	-	-
8	15.3	3	18.2	6	16.5	2	16.5	2	16.5
9	18.5	2	19.2	6	18.0	2	18.0	2	18.0
10	18.0	1	20.0	1	17.0	1	17.0	1	17.0







true for D. schødleri of Big Island Lake. Low temperature tended to increase the duration of each embryonic stage, and hence the embryonic period. Low temperatures also increased the adult instar duration, and hence the brooding period.

As mentioned previously, young D. schødleri when released from their mother's brood pouch, usually are in a developmental stage similar to the 53 hour stage in vitro (Fig. 42-29'). This does not necessarily mean that the brooding period is synonymous with embryonic period. The embryonic period is usually completed by the time the young are liberated; and occasionally the young are retained in the brood pouch for some time after the embryonic period has ended.

#### Discussion of Factors Relating to

#### Egg Production

Some Daphnia may produce resting eggs and ephippia without the presence of males. These unfertilized resting eggs may be broken up and reabsorbed in the ovary, and the ephippium thrown off, but of course the ephippium will be empty (Scharfenberg, 1910; Berg, 1931); sometimes the unfertilized resting eggs are transferred to the brood pouch but are destroyed and expelled before the ephippium completes its formation (Scharfenberg, 1914; Berg, 1931); in other cases the unfertilized resting eggs are transferred to the brood pouch (ephippium), and the resting eggs subsequently develop into viable young (Oloffson, 1918; Banta, 1926; Schrader, 1926; Poulsen, 1940a & b; Edmondson, 1955).

In my study, several D. schødleri females reared at  $5 \pm 1$  C produced resting eggs without the prior appearance of males; and these resting eggs were successfully deposited into the modified brood pouch. In some cases, one or both of the resting eggs were destroyed and expelled before the ephippium completed its formation. However, quite often, the resting eggs



remained intact and were thrown off with the ephippium during the molt. These ephippia with the unfertilized resting eggs were kept in a small beaker with fresh culture medium at  $20 \pm 1$  C. They did not hatch. It seems that under suitable stimulation (probably low temperature and perhaps short photoperiod) that D. schødleri females are capable of producing ephippial eggs and ephippia without the presence of males; but ephippial eggs must be fertilized if they were to produce viable young.

It has been recognized for a long time that the stimuli involved in male production are apparently quite different from the stimuli involved in the formation of ephippial eggs (Woltereck, 1911; Scharfenberg, 1914; Banta and Brown, 1929a & b; Berg, 1931; Banta et al., 1939). In my study of D. schødleri, ephippia were produced by females kept in a refrigerator with temperature maintained at  $5 \pm 1$  C; however, no males were found among the offspring produced by these females. It appears that low temperature and possibly short photoperiod (resulting from the refrigerator door being opened and closed) is capable of inducing ephippial production among individually raised females of D. schødleri; but these stimuli are not capable of inducing the production of male offspring. This supports the view that stimuli involved in male production and the formation of ephippial eggs are apparently quite different.





## SUMMARY

1. The life history of Daphnia schødleri was investigated by combining field and laboratory studies. Daphnia used for laboratory experiments were maintained at temperatures of 22-29.4 C,  $20 \pm 1$  C, and  $5 \pm 1$  C. The population of D. schødleri in Big Island Lake was studied from June 1966 to July 1967.
2. D. schødleri in Big Island Lake overwintered in the resting egg stage. Hatching takes place in late spring and by May parthenogenetic reproduction is taking place.
3. There were two periods of sexual reproduction; a major period in June and July, and a minor period in September. The major period of sexual reproduction occurred when the population was exhibiting maximum numbers.
4. Egg production (average number of eggs per parthenogenetic female per brood) varied seasonally. The average number of eggs per parthenogenetic female diminished immediately before the beginning of the sexual periods. This decrease in brood size was not due to a decrease in the average size of the egg-bearing females.
5. Average brood size of female D. schødleri ranged from 2.50 to 29.90 eggs; the over-all average brood size during the study period was 7.96 eggs.
6. Both parthenogenetic and gamogenetic (ephippial) females exhibited seasonal size variations. Ehippial females were generally smaller than parthenogenetic females.
7. In Big Island Lake, the number of parthenogenetic eggs produced per brood was directly proportional to the size of the female.
8. The sizes (volumes) of the parthenogenetic eggs of D. schødleri varied seasonally and was influenced by variations in the body size of females





carrying these eggs.

9. The number of pre-adult instars of D. schødleri in culture varied from 4-7. Low temperature increased the number of pre-adult instars.
10. At temperatures fluctuating between 22 and 29.4 C the average longevity of D. schødleri was 40.97 days (17.65 instars) for males, and 36.42 days (18.21 instars) for females. At  $20 \pm 1$  C the average longevity for females was 51.93 days (20.78 instars).
11. The initial size of the young females influenced the instar in which they reached sexual maturity; as the initial size increased the animals tended to become mature in earlier instars.
12. The growth of D. schødleri was studied at different temperatures. The growth curve was sigmoid. The growth increment increased during early instars, and then decreased in later instars. The greatest growth increment did not always occur at the end of the adolescent instar; it sometimes occurred at the end of the pre-adolescent instar or even earlier.
13. In general the duration of each instar increased with the age of the female in question.
14. The number of young produced during each adult instar was highest in the early broods, and the number decreased in later broods.
15. Egg production and size of females were positively correlated in the first adult instar.
16. Frequency of molting, duration of embryonic development, growth rate, and physiological life span of D. schødleri were all influenced by temperature. Low temperatures increased the duration of embryonic development and life span, and decreased the frequency of molting and impeded growth rate. Low temperature also delayed the onset of sexual maturity of female D. schødleri.



17. The size of the young produced varied with maternal age; young were larger during the early broods and smaller in later broods.
18. The size of young produced varied with temperature. The young produced at low temperatures were larger than those produced at higher temperature.
19. A description was given of the sequence of events in the course of embryonic development in vitro, as observed under microscope.
20. The length of the brooding periods, i.e. the time between the deposition of eggs and their subsequent release as young, varied with the length of the maternal instars.





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Phytoplankton of Big Island Lake during July 1966 to July 1967. x indicates present in sample.

	1966							
	July 27	Aug. 3	Aug. 17	Aug. 26	Oct. 16	Nov. 27	Dec. 18	Jan. 5
<b>Cyanophyta</b>								
<u>Microcystis flos-aquae</u> (Wittr.) Kirchn.	x	x	x	x	x	x	x	x
<u>Microcystis aeruginosa</u> Kuetz.	x	x	x	x	x	x	x	x
<u>Anabaena flos-aquae</u> (Lyngb.) Breb.	x	x	x	x	x	x	x	x
<u>Aphanizomenon flos-aquae</u> (L.) Ralfs	x	x	x	x	-	-	x	x
<u>Chroococcus</u> sp.	x	x	x	x	x	-	x	-
<u>Oscillatoria</u> sp.	-	-	-	-	-	-	-	-
<u>Merismopedia</u> sp.	-	-	x	x	x	-	-	x
<b>Chlorophyta</b>								
<u>Pediastrum boryanum</u> (Turp.) Menegh.	x	x	x	x	x	x	x	x
<u>Pediastrum duplex</u> Meyen	x	x	x	x	x	x	x	x
<u>Scenedesmus</u> spp.	x	x	x	x	x	x	x	x
<u>Eudorina</u> sp.	-	-	-	-	-	-	-	-
<u>Volvox</u> sp.	-	-	-	-	-	-	-	-
<u>Gloeocystis</u> sp.	-	-	-	-	-	-	-	-
<u>Ankistrodesmus</u> sp.	-	-	-	-	x	-	-	-
<u>Coelastrum</u> sp.	-	-	-	-	-	-	-	-
<u>Asterococcus</u> sp.	-	-	-	-	-	-	-	-
<u>Staurastrum</u> sp.	x	x	x	x	x	x	x	x
<u>Cosmarium</u> sp.	-	-	x	-	x	-	-	-
<u>Closterium</u> spp.	x	x	x	x	x	-	-	-
<u>Xanthidium</u> sp.	-	-	-	-	-	-	x	-
<u>Spirogyra</u> sp.	-	-	-	-	x	-	-	-
<u>Oedogonium</u> sp.	-	x	-	-	-	-	-	x
<b>Chrysophyta</b>								
<u>Cyclotella</u> sp.	-	x	x	x	-	x	x	x
<u>Melosira</u> sp.	x	x	x	x	x	x	x	x
<u>Fragilaria</u> sp.	-	-	-	-	-	x	-	-
<u>Asterionella</u> sp.	-	-	-	-	-	x	-	-
<u>Synedra</u> sp.	-	x	x	x	x	x	x	x
<u>Cymbella</u> sp.	-	-	-	-	-	-	x	x
<u>Surirella</u> sp.	-	-	-	-	-	-	-	-
<u>Cymatopleura</u> sp.	-	-	-	x	-	x	x	x
<u>Navicula</u> sp.	x	x	x	x	x	x	x	x
<u>Gomphonema</u> sp.	x	x	x	-	-	x	x	x
<u>Pinnularia</u> sp.	x	-	-	-	-	-	x	-
<u>Eunotia</u> sp.	-	-	-	-	-	-	-	-
<u>Amphora</u> sp.	-	-	-	-	-	-	x	x
<u>Frustulia</u> sp.	-	-	-	-	-	-	-	-
<b>Pyrrophyta</b>								
<u>Ceratium hirundinella</u> (O.F.M.) Dujardin	-	-	-	-	-	x	x	-



		1967							
		Mar.	Apr.	May	May	June	June	June	July
		19	9	13	27	3	13	24	3
Cyanophyta									
<u>Microcystis flos-aquae</u> (Wittr.) Kirchn.	x	x	x	x	x	x	x	x	x
<u>Microcystis aeruginosa</u> Kuetz.	x	x	x	x	x	x	x	x	x
<u>Anabaena flos-aquae</u> (Lyngb.) Breb.	x	x	x	-	x	x	x	x	x
<u>Aphanizomenon flos-aquae</u> (L.) Ralfs	x	-	x	-	-	x	x	x	x
<u>Chroococcus</u> sp.	x	-	x	x	x	x	x	x	x
<u>Oscillatoria</u> sp.	-	-	-	-	x	x	-	-	-
<u>Merismopedia</u> sp.	-	-	-	-	x	x	-	-	-
Chlorophyta									
<u>Pediastrum boryanum</u> (Turp.) Menegh.	x	x	x	x	x	x	x	x	x
<u>Pediastrum duplex</u> Meyen	x	x	x	x	x	x	x	x	x
<u>Scenedesmus</u> spp.	x	x	-	x	x	x	x	x	x
<u>Eudorina</u> sp.	-	-	x	-	-	-	-	-	-
<u>Volvox</u> sp.	-	-	x	-	-	-	-	-	-
<u>Gloeocystis</u> sp.	-	-	-	x	x	-	-	-	x
<u>Ankistrodesmus</u> sp.	-	-	-	x	-	-	-	-	-
<u>Coelastrum</u> sp.	-	-	-	-	-	-	x	x	x
<u>Asterococcus</u> sp.	-	-	-	-	-	x	-	-	x
<u>Staurastrum</u> sp.	x	x	-	-	x	x	-	-	-
<u>Cosmarium</u> sp.	-	-	-	-	-	-	-	-	-
<u>Closterium</u> spp.	-	-	-	-	x	x	x	x	x
<u>Xanthidium</u> sp.	-	-	-	-	-	-	-	-	-
<u>Spirogyra</u> sp.	-	-	-	-	-	-	-	-	-
<u>Oedogonium</u> sp.	x	x	-	-	-	-	-	-	-
Chrysophyta									
<u>Cyclotella</u> sp.	x	x	-	-	x	-	x	x	x
<u>Melosira</u> sp.	x	x	x	x	x	x	x	x	x
<u>Fragilaria</u> sp.	-	-	-	-	-	x	x	x	x
<u>Asterionella</u> sp.	x	-	x	-	-	-	-	-	-
<u>Synedra</u> sp.	x	x	-	-	x	x	x	x	x
<u>Cymbella</u> sp.	x	x	-	-	-	x	-	-	-
<u>Surirella</u> sp.	-	-	x	x	x	-	-	-	-
<u>Cymatopleura</u> sp.	x	x	x	x	x	-	-	-	-
<u>Navicula</u> sp.	x	x	x	x	x	x	x	x	x
<u>Gomphonema</u> sp.	x	x	x	x	x	x	-	-	-
<u>Pinnularia</u> sp.	-	x	x	-	x	x	-	-	-
<u>Eunotia</u> sp.	-	x	-	x	x	-	-	-	-
<u>Amphora</u> sp.	x	x	-	-	x	-	-	-	-
<u>Frustulia</u> sp.	x	x	x	x	x	-	-	-	-
Pyrrophyta									
<u>Ceratium hirundinella</u> (O.F.M.) Dujardin	x	-	-	-	-	-	-	-	-



## Appendix 2A.

Mean values of total length, carapace length, and height for each instar of the eight female Daphnia schodleri primiparous in the sixth instar and living for at least 20 instars at temperatures fluctuating from 22-29.4 C.

Instar	Total length mm	Carapace length mm	Height mm	Number of animals
1	0.5203	0.3951	0.2730	8
2	0.6622	0.4721	0.3331	8
3	0.8531	0.6244	0.4526	8
4	1.1164	0.8137	0.5403	8
5	1.4016	1.0510	0.7414	8
6	1.6923	1.2980	0.9409	8
7	1.8659	1.4414	1.0217	8
8	2.0857	1.6213	1.1562	8
9	2.2202	1.7245	1.2545	8
10	2.3389	1.8147	1.3203	8
11	2.4046	1.8708	1.3573	8
12	2.4745	1.9286	1.4020	8
13	2.5322	1.9683	1.4259	8
14	2.5947	2.0105	1.4610	8
15	2.6508	2.0560	1.5060	8
16	2.6804	2.0812	1.5299	8
17	2.7101	2.1060	1.5438	8
18	2.7584	2.1405	1.5645	8
19	2.8036	2.1722	1.5941	8
20	2.8263	2.1893	1.6153	8
21	2.8291	2.1867	1.6088	6
22	2.8703	2.1883	1.6153	6
23	2.8750	2.2250	1.6432	5
24	2.8724	2.2198	1.6510	5
25	2.8803	2.2230	1.6357	4
26	2.8819	2.2287	1.6567	4
27	2.9283	2.2707	1.6792	3







## Appendix 2B.

Mean growth increments for the animals of Appendix 2A.

Instar	Total length mm	Carapace length mm	Height mm
1	0.1419	0.0770	0.0601
2	0.1909	0.1523	0.1195
3	0.2633	0.1893	0.0877
4	0.2852	0.2373	0.2011
5	0.2907	0.2470	0.1995
6	0.1736	0.1434	0.0808
7	0.2198	0.1799	0.1345
8	0.1345	0.1032	0.0983
9	0.1187	0.0902	0.0658
10	0.0657	0.0561	0.0370
11	0.0699	0.0578	0.0447
12	0.0577	0.0397	0.0239
13	0.0625	0.0422	0.0351
14	0.0561	0.0455	0.0450
15	0.0296	0.0252	0.0239
16	0.0297	0.0248	0.0139
17	0.0483	0.0345	0.0207
18	0.0452	0.0317	0.0296
19	0.0227	0.0171	0.0212
20	0.0028	-0.0026	-0.0065
21	0.0412	0.0016	0.0065
22	0.0047	0.0367	0.0279
23	-0.0026	-0.0052	0.0078
24	0.0079	0.0032	-0.0153
25	0.0016	0.0057	0.0210
26	0.0464	0.0420	0.0225



## Appendix 3A.

Mean values of total length, carapace length, and height for each instar of the eight male Daphnia schodleri that lived for at least 18 instars at temperatures fluctuating from 22-29.4 C.

Instar	Total length mm	Carapace length mm	Height mm	Number of animals
1	0.7633	0.5513	0.3904	8
2	0.8503	0.6094	0.4558	8
3	1.0640	0.8023	0.5342	8
4	1.2269	0.9238	0.6057	8
5	1.3362	1.0168	0.6618	8
6	1.4056	1.0863	0.6943	8
7	1.4540	1.1310	0.7284	8
8	1.4938	1.1692	0.7564	8
9	1.5377	1.2025	0.7824	8
10	1.5844	1.2358	0.7971	8
11	1.6335	1.2756	0.8231	8
12	1.6758	1.3114	0.8438	8
13	1.7063	1.3317	0.8556	8
14	1.7331	1.3480	0.8747	8
15	1.7717	1.3695	0.8929	8
16	1.7932	1.3975	0.9088	8
17	1.8269	1.4219	0.9226	8
18	1.8497	1.4422	0.9364	8
19	1.8827	1.4746	0.9597	7
20	1.9105	1.4793	0.9663	6
21	1.9281	1.5137	0.9750	4
22	1.9589	1.5364	0.9848	4
23	1.9898	1.5576	1.0108	4
24	1.9646	1.5389	0.9913	2
25	1.9971	1.5730	1.0124	2
26	2.0313	1.6088	1.0400	1



## Appendix 3B.

Mean growth increments for males of Appendix 3A.

Instar	Total length mm	Carapace length mm	Height mm
1	0.0870	0.0581	0.0654
2	0.2137	0.1929	0.0784
3	0.1629	0.1215	0.0715
4	0.1093	0.0930	0.0561
5	0.0694	0.0695	0.0325
6	0.0484	0.0447	0.0341
7	0.0398	0.0382	0.0280
8	0.0439	0.0333	0.0260
9	0.0464	0.0333	0.0147
10	0.0491	0.0398	0.0260
11	0.0423	0.0358	0.0207
12	0.0305	0.0203	0.0118
13	0.0268	0.0163	0.0191
14	0.0386	0.0215	0.0182
15	0.0215	0.0280	0.0159
16	0.0337	0.0244	0.0138
17	0.0228	0.0203	0.0138
18	0.0330	0.0324	0.0233
19	0.0278	0.0047	0.0066
20	0.0176	0.0344	0.0087
21	0.0308	0.0227	0.0098
22	0.0309	0.0212	0.0260
23	-0.0252	-0.0187	-0.0195
24	0.0325	0.0341	0.0211
25	0.0342	0.0358	0.0276





Mean length and mean length increment for each instar of female Daphnia  
schødleri at 20 ± 1 C.

Instar.	Mean length mm	Mean length increment mm	Number of animals
1	0.6533	0.2171	31
2	0.8704	0.3040	31
3	1.1744	0.3645	31
4	1.5389	0.3184	31
5*	1.8573	0.2095	31
6	2.0668	0.1221	31
7	2.1889	0.1433	31
8	2.3322	0.0900	29
9	2.4222	0.0726	29
10	2.4948	0.0760	29
11	2.5708	0.0777	29
12	2.6485	0.0710	29
13	2.7195	0.0500	29
14	2.7695	0.0375	27
15	2.8070	0.0294	27
16	2.8364	0.0517	27
17	2.8881	0.0325	25
18	2.9206	0.0360	25
19	2.9566	0.0511	24
20	3.0077	0.0355	22
21	3.0432	0.0270	19
22	3.0702	0.0291	19
23	3.0993	0.0520	19
24	3.1513	0.0250	19
25	3.1763	0.0175	18
26	3.1938	0.0252	18
27	3.2190	0.0169	17
28	3.2359	0.0245	17
29	3.2604	0.0178	16
30	3.2782	0.0355	15
31	3.3137	0.0368	15
32	3.3505	0.0128	15
33	3.3633	0.0319	13
34	3.3952	0.0271	12
35	3.4223	0.0307	11
36	3.4530	0.0226	11
37	3.4756	0.0182	10
38	3.4938	0.0195	8
39	3.5133	-0.0033	5
40	3.5100	-0.0162	2
41	3.4938	-	2

\* indicates first adult instar.



Appendix 5. Mean length and mean length increment for each instar of Daphnia schöpleri primiparous in different instars at  $5 \pm 1$  C.

Instar	Sixth instar				Seventh instar				Eighth instar			
	Mean length mm	Mean length increment mm	Number of individuals	Mean length mm	Mean length increment mm	Number of individuals	Mean length mm	Mean length increment mm	Number of individuals	Mean length mm	Mean length increment mm	Number of individuals
1	0.7001	0.1264	6	0.6435	0.1235	3	0.6188	0.0952	10	0.6338	0.1088	2
2	0.8265	0.1511	6	0.7670	0.1961	3	0.7140	0.1768	10	0.7425	0.1755	2
3	0.9776	0.3387	6	0.9631	0.2665	3	0.8908	0.2630	10	0.9181	0.2113	2
4	1.3163	0.3682	6	1.2296	0.3078	3	1.1538	0.3328	10	1.1294	0.2925	2
5	1.6845	0.3906	6	1.5383	0.2622	3	1.4866	0.3386	10	1.4219	0.3004	2
6	2.0751	0.2974	6	1.8005	0.1105	3	1.8252	0.3741	10	1.7225	0.2590	2
7	2.3725	0.1733	4	1.9110	0.1040	3	2.1993	0.2069	10	1.9825	0.3250	2
8	2.5458	0.1332	3	2.0150	0.0585	3	2.4042	0.1591	8	2.3075	0.1788	2
9	2.6790	0.0998	3	2.0735	-0.0162*	3	2.5653	0.2311	6	2.4803	0.1767	2
10	2.7788	0.2112	2	2.0573*	0.0877	1	2.7964	0.0636	5	2.6050	0.0650	2
11	2.9900	-	1	2.1450	0.0163	1	2.8600	-	1	2.7300	-	1
12	-	-	-	2.1613	-	1	-	-	-	-	-	-

# Indicates females that produced ephippia.

\* This reduction in mean length is due to the death of larger animals.



## Appendix 6.

Mean length for Daphnia schødleri of different ages (in days) primiparous in the fifth instar at  $20 \pm 1$  C.

Age in days	Mean length mm	Age in days	Mean length mm
0*	0.6533	46.4	3.0702
0.95	0.8704	49.15	3.0993
1.99	1.1744	51.89	3.1513
3.01	1.5389	54.66	3.1763
4.42	1.8573	57.50	3.1938
6.63	2.0068	60.45	3.2190
8.86	2.1889	63.40	3.2359
11.08	2.3322	66.31	3.2604
13.41	2.4222	69.36	3.2782
15.76	2.4948	72.19	3.3137
18.08	2.5708	75.09	3.3505
20.49	2.6485	77.92	3.3633
22.96	2.7195	80.78	3.3952
25.44	2.7695	83.63	3.4223
27.96	2.8070	86.52	3.4530
30.60	2.8364	89.55	3.4756
33.14	2.8881	92.52	3.4938
35.72	2.9206	95.84	3.5133
38.31	2.9566	99.08	3.5100
40.93	3.0077	104.32	3.4938
43.62	3.0432		

\* indicates the time when animals were released from their mother's brood chamber.





Appendix 7.

Mean length of Daphnia schødlerei primiparous in different instars at  $5 \pm 1$  C.

Sixth instar			Seventh instar			Eighth instar			Seventh instar#		
Age in days	Mean length mm	Age in days	Mean length mm	Age in days	Mean length mm	Age in days	Mean length mm	Age in days	Mean length mm	Age in days	Mean length mm
0*	0.7001	0*	0.6188	0*	0.6338	0*	0.6435	0*	0.6435	0*	0.6435
9	0.8265	10.7	0.7140	9.5	0.7426	8.3	0.7670	8.3	0.7670	8.3	0.7670
17.7	0.9776	18.8	0.8908	15	0.9181	14.4	0.9631	14.4	0.9631	14.4	0.9631
25.9	1.3163	25.5	1.1538	22	1.1294	20.6	1.2296	20.6	1.2296	20.6	1.2296
33.1	1.6845	32	1.4866	29	1.4219	27.9	1.5383	27.9	1.5383	27.9	1.5383
41.6	2.0751	39.6	1.8252	36	1.7225	35.9	1.8005	35.9	1.8005	35.9	1.8005
54.8	2.3725	49	2.1993	44.5	1.9825	48.6	1.9110	48.6	1.9110	48.6	1.9110
70.8	2.5458	65	2.4062	55	2.3075	59.3	2.0150	59.3	2.0150	59.3	2.0150
86	2.6790	83.7	2.5653	72	2.4863	69.6	2.0735	69.6	2.0735	69.6	2.0735
105.5	2.7788	103.1	2.7964	90.5	2.6650	83.1	2.0573	83.1	2.0573	83.1	2.0573
-	-	-	-	-	-	111.1	2.1613	111.1	2.1613	111.1	2.1613

# indicates females that produced ephippia.

\* represents the time when animals were released from their mother's brood chamber.







# Appendix 8B.

Mean growth of pre-adult instars and mean growth of first adult instar for femal Daphnia schødleri at temperature of  $20 \pm 1$  C.

Instar in which first eggs were laid	Length (mm) in instar number								Number of animals
	1	2	3	4	5	6	7	8	
5	0.6812	0.8583	1.2061	1.4856	1.7638	1.8850	-	-	7
5	0.6419	0.8613	1.1944	1.5275	1.8242	1.9663	-	-	4
5	0.6412	0.8538	1.1525	1.5109	1.8099	1.9971	-	-	33
5	0.6266	0.8239	1.1131	1.4424	1.7713	2.0166	-	-	4
5	0.6286	0.8330	1.1250	1.4596	1.8164	2.0495	-	-	7
6	0.7118	0.8905	1.2025	1.3813	1.5925	1.7648	1.9175	-	1
6	0.6074	0.7641	1.0010	1.3120	1.5883	1.8135	1.9565	-	7
6	0.6263	0.7735	1.0293	1.3432	1.6575	1.8762	1.9175	-	3
6	0.5395	0.6685	0.8538	1.1229	1.4778	1.7251	1.9120	-	6
6	0.5759	0.7150	0.9334	1.2067	1.5015	1.8291	2.0017	-	10
7	0.4875	0.6338	0.7475	0.9425	1.2025	1.5275	1.7550	1.8200	1





## Appendix 8C.

Mean growth increments of pre-adult instars and mean growth increments of first adult instar as percentage of initial length for female Daphnia schødleri.

Culture temperature	Instar in which first eggs were laid	Increment as percentage initial length						
		1	2	3	4	5	6	7
22-29.4 C	5	31	50	51	67	50	-	-
	5	32	47	54	68	68	-	-
	6	21	30	55	55	52	-	-
	6	27	39	48	60	53	-	-
	6	28	34	41	56	38	-	-
	6	21	37	48	51	57	28	-
20 $\pm$ 1 C	5	26	51	41	41	18	-	-
	5	34	52	52	46	22	-	-
	5	33	47	56	47	29	-	-
	5	31	46	53	53	39	-	-
	5	33	47	53	57	37	-	-
	6	25	44	25	30	24	22	-
	6	26	39	51	46	37	24	-
	6	24	41	50	50	35	7	-
	6	24	34	50	66	46	35	-
	6	24	38	48	51	57	30	-
	7	30	23	40	53	67	47	13



## Appendix 9A.

Mean growth for first six instars of male Daphnia schødleri at temperatures fluctuating from 22-29.4 C.

Length (mm) in instar number						Number of animals
1	2	3	4	5	6	
0.7576	0.8437	1.0631	1.2093	1.3150	1.3887	14
0.7800	0.8631	1.0563	1.2513	1.3488	1.4138	1
0.7280	0.8362	0.9939	1.2067	1.3319	1.4017	4

## Appendix 9B.

Mean growth increments as percentage of initial length for first five instars of male Daphnia schødleri at temperatures fluctuating from 22-29.4 C.

Increment as percentage initial length					Number of animals
1	2	3	4	5	
11	29	19	14	10	14
10	25	25	13	8	1
15	22	29	17	10	4





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